F 601
43

American Journal of

DEC 6 1946

Veterinary Research

CONTENTS

Studies on Brucellosis in Swine. III. Brucella Infection in the Boar..... 379
L. M. Hutchings and F. N. Andrews

Research Fellowships Available..... 384

Studies on Brucellosis in Swine. IV. Semen Quality in Brucella-Infected Boars.. 385
F. N. Andrews and L. M. Hutchings

Brucellosis in Swine. V. Reproduction Studies with Naturally Infected Sows and Boars..... 388
L. M. Hutchings, A. L. Delez, and C. R. Donham

Comparative Growth of the Sheep and Bovine Animal During Prenatal Life..... 395
W. W. Green

The Relation of Riboflavin to Equine Periodic Ophthalmia..... 403
T. C. Jones, Thomas O. Roby, and Fred D. Maurer

Experimental Treatment of Trichomonas Foetus Infection in Bulls..... 417
David E. Bartlett

Bacteriopathology of Infectious Bovine Pyelonephritis..... 432
E. S. Feenstra and F. Thorp, Jr.

The Histopathology of a Focal Hepatitis and of Its Termination ("Sawdust" and "Telang" Liver) in Cattle..... 437
Robert Getty

Average Levels of Various Constituents, Physical Properties, and Formed Elements of the Blood of Cows on Pasture..... 450
Werner Braun

The Histopathology of Panleucopenia (Agranulocytosis) in the Domestic Cat..... 455
Wayne H. Riser

The Effect of Certain Chemical Agents on the Virus of Infectious Bronchitis of Chickens..... 466
Charles H. Cunningham and H. O. Stuart

Reaction of the Developing Chicken Embryo and the Chorioallantoic Membrane to Inoculation with Various Strains of Mycobacterium Paratuberculosis (Johne's Bacillus)..... 470
Abram B. Stavitsky and John D. Beck

Two Examples of Terata in the Caudal Region of Domestic Poultry..... 477
I. L. Kosin

Recovery of Viable Anthrax Spores from Vapors of Heated Anthrax Spore Suspensions and from the Distillate from Anthrax Spore Suspensions..... 481
C. D. Stein and Herbert Rogers

The Action of Photosensitising Agents Isolated from Buckwheat..... 486
Simon H. Wender

Reproductive Failure and Agalactia in Ergotized Female Rats..... 490
A. W. Nordskog

Index..... 498

A Publication of the American Veterinary Medical Association

Volume VII OCTOBER, 1946 Number 25

Consecutive order of appearance, rather than identity with any given volume, is the plan adopted for numbering the issues of the American Journal of Veterinary Research. Following are the volume and issue numbers published to date.

VOL. 1, No. 1—OCTOBER, 1940

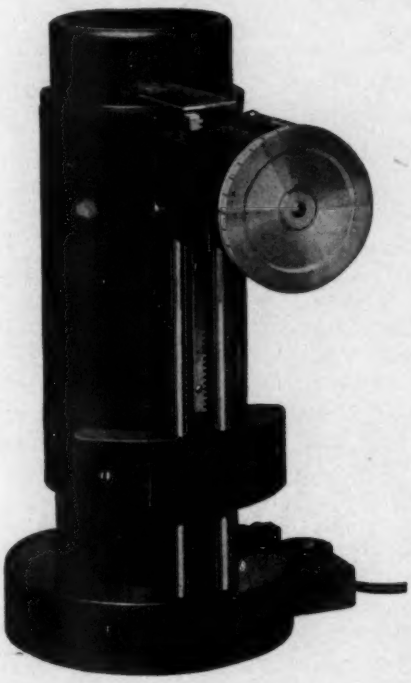
VOL. II, No. 2—JANUARY, 1941	VOL. IV, No. 10—JANUARY, 1943
VOL. II, No. 3—APRIL, 1941	VOL. IV, No. 11—APRIL, 1943
VOL. II, No. 4—JULY, 1941	VOL. IV, No. 12—JULY, 1943
VOL. II, No. 5—OCTOBER, 1941	VOL. IV, No. 13—OCTOBER, 1943
VOL. III, No. 6—JANUARY, 1942	VOL. V, No. 14—JANUARY, 1944
VOL. III, No. 7—APRIL, 1942	VOL. V, No. 15—APRIL, 1944
VOL. III, No. 8—JULY, 1942	VOL. V, No. 16—JULY, 1944
VOL. III, No. 9—OCTOBER, 1942	VOL. V, No. 17—OCTOBER, 1944

VOL. VI, No. 18—JANUARY, 1945
VOL. VI, No. 19—APRIL, 1945
VOL. VI, No. 20—JULY, 1945
VOL. VI, No. 21—OCTOBER, 1945
VOL. VII, No. 22—JANUARY, 1946
VOL. VII, No. 23—APRIL, 1946 (PART 1).
VOL. VII, No. 23—APRIL, 1946 (PART 2)
VOL. VII, No. 24—JULY, 1946
VOL. VII, No. 25—OCTOBER, 1946

COPYRIGHT 1946 BY AMERICAN VETERINARY MEDICAL ASSOCIATION

Published quarterly at 600 S. Michigan Ave., Chicago, Ill., by the American Veterinary Medical Association. Entered as Second-Class Matter January 31, 1941, at the Post Office at Chicago, Illinois, under the Act of March 3, 1879. Subscription price: $4.00 per year for United States, Canada, and countries affiliated with the Pan American Postal Union; foreign countries, $4.30. Members of the American Veterinary Medical Association may subscribe to the American Journal of Veterinary Research for $2.50 per year added to the annual dues of $7.00. Single copies $1.25. Address all correspondence to American Veterinary Medical Association.

Printed in the U. S. A.



For measuring the mean erythrocyte diameter by the light diffraction method

Haden-Hausser Erythrocytometer

DIFFRACTOMETER

The Haden-Hausser Erythrocytometer employs the diffraction principle for measuring the mean diameter of a large number of corpuscles. This principle is based upon the phenomenon that when a beam of light passes the edges of an obstacle or aperture some of the waves are deflected, the degree depending upon the length of the wave. Since white light is composed of waves of varying lengths each of which is characterized by a different color, a series of colored rings will result. If many obstacles or apertures are spaced so closely as to minimize the undiffracted rays they will superimpose upon each other a large number of spectra so as to be easily visible to the unaided eye. A blood film fulfills all these requirements and since the size of the spectrum varies inversely with the size of the obstacle the diffraction principle can be employed to measure the mean diameter of a large number of corpuscles.

The instrument can be used with three types of specimens: fixed or stained films mounted in physiological salt solution of water, in suspension, and with unfixed films. In addition to measuring the mean diameter of the corpuscles of the specimen the instrument can also be used to record anisocytosis.

A-2529/A. Haden-Hausser Erythrocytometer complete as described above with chamber for measuring corpuscles in suspension, directions for use, charts and formulae. ***Each*** **$50.00**

A-2529. Same as above, but with case. " **60.00**

JUST OUT! New 32 page booklet on Blood Testing Instruments. Write for your free copy.

CLAY-ADAMS CO INC.

44 EAST 23rd STREET, NEW YORK 10, N. Y.

Editors

L. A. MERILLAT — J. G. HARDENBERGH
R. C. KLUSSENDORF — HELEN S. BAYLESS

Committee on Journal

C. C. HASTINGS, B. T. SIMMS, and W. A. HAGAN

Research Council

Anatomy and Histology

L. E. ST. CLAIR, D.V.M., Professor of Veterinary Anatomy and Histology, College of Veterinary Medicine, University of Illinois, Urbana. (1949).

Bacteriology (Immunology and Biologic Therapy)

EDWARD RECORDS, V.M.D., Research Professor of Veterinary Science, University of Nevada, Reno. (1947).

Biochemistry and Animal Nutrition

GEORGE H. HART, V.M.D., M.D., Head, Division of Animal Husbandry, University of of California, Davis. (1947).

Large Animal Medicine

JAMES FARQUHARSON, D.V.M., Professor of Surgery and Clinics, Division of Veterinary Medicine, Colorado State College, Ft. Collins. (1947).

Large Animal Surgery

W. F. GUARD, D.V.M., Professor of Surgery and Obstetrics, College of Veterinary Medicine, The Ohio State University, Columbus. (1948).

Parasitology

E. W. PRICE, D.V.M., M.S., Ph.D., Assistant Chief, Zoölogical Division, Bureau of Animal Industry, Beltsville Research Center, Beltsville, Md. (1948).

Pathology

E. T. HALLMAN, D.V.M. (*Chairman*), Professor of Pathology, School of Veterinary Medicine, Michigan State College, East Lansing. (1947).

Physiology and Pharmacology

H. H. DUKES, B.S., D.V.M., M.S. (*Secretary*), Professor of Veterinary Physiology, New York State Veterinary College, Cornell University, Ithaca. (1947).

Poultry Pathology

C. A. BRANDLY, D.V.M., Professor of Veterinary Medicine and Agricultural Bacteriology, University of Wisconsin, Madison. (1948).

Small Animal Medicine

C. P. ZEPP, SR., D.V.M., 136 West 53rd St., New York, N.Y. (1949).

Small Animal Surgery

C. F. SCHLOTTHAUER, D.V.M., Associate Professor, Division of Experimental Medicine, Mayo Foundation, Rochester, Minn. (1946).

Veterinary Hygiene

ANDREW L. MACNABB, B.V.Sc., Principal, Ontario Veterinary College, Guelph. (1949).

Virus Diseases

R. A. KELSER, D.V.M., A.M., Ph.D. (*Vice-Chairman*), Dean School of Veterinary Medicine, University of Pennsylvania, Philadelphia. (1949).

X-Ray

M. A. EMMERSON, D.V.M., M.S., Dr. Med. vet. (Zurich), Professor of Veterinary Obstetrics, Division of Veterinary Medicine, Iowa State College, Ames. (1948).

Member-at-Large

HADLEIGH MARSH, D.V.M., Veterinary Pathologist, Agricultural Experiment Station, Bozeman, Mont. (1948).

The Treatment of

ATONIC INDIGESTION IN CATTLE

with

LENTIN

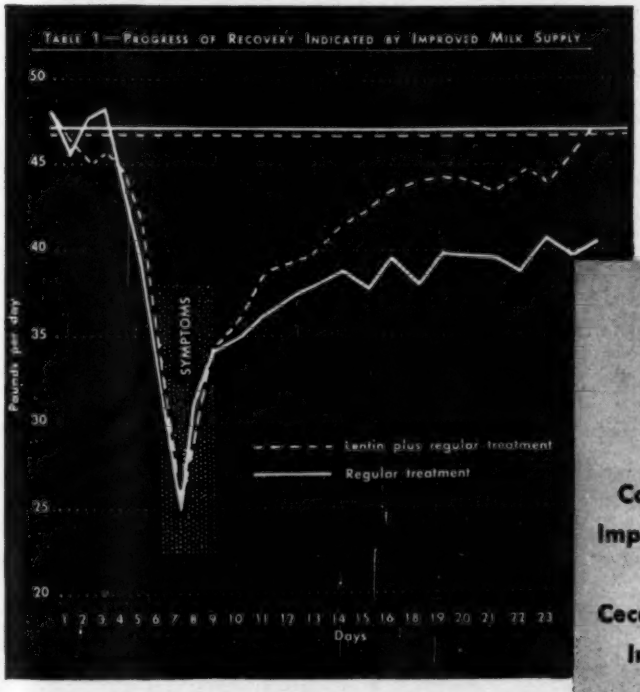
Reg. U. S. Pat. Off.

LENTIN has been reported to be useful in the treatment of atonic indigestion in dairy cattle. In a report covering 20 cases, the author* states that his experiences were highly satisfactory, and that the results were spectacular in severe and neglected cases because the animals began to show glandular activity and rumen movements soon after injection.

In a comparative study, milk production increased more promptly in 8 cows treated with Lentin than in an equal number of controls receiving routine treatment consisting of tartar emetic (90 grains) and 1 ounce each of ginger and gentian, and water if dehydration was present. Even more important was the fact that, in the Lentin-treated animals, the level of milk production returned to that existing prior to the development of the atony. The superior results obtained with Lentin are shown in the accompanying graph.

For best results, Lentin with supplementary therapy should be given promptly, because withholding treatment 24 hours after symptoms developed delayed recovery by about 7 days.

Lentin is distributed by licensed veterinary supply houses.



*Holm, G. C., A preliminary report of the use of Lentin in atonic indigestion of dairy cattle, Vet. Med. 39: 414-416, Nov. 1944.

LENTIN

FOR THE TREATMENT OF

Constipation — **Atony of Bowel**
Impaction — **Gastro-intestinal Catarrh**
Tympanites — **Dyspepsia**
Cecal and Colonic Impaction in Horses
Impaction and Atony of Rumen
Cattle and Sheep Choke
(esophageal obstruction)

MERCK & CO., Inc. *Manufacturing Chemists* **RAHWAY, N. J.**

DIFCO

INGREDIENTS FOR
Bacteriological Culture Media

EACH of these Culture Media Ingredients, Difco, is standardized to meet the highest requirements of suitability and usefulness for the purpose for which it is to be employed.

BACTO-PEPTONE

BACTO-PEPTONE is most widely used in the preparation of routine culture media. It is rich in readily available forms of nitrogen, and in a one per cent solution it is sparklingly clear with a reaction of pH 7.0.

BACTO-TRYPTONE

BACTO-TRYPTONE is an excellent peptone for use in media for detection of indol production by bacteria. With this peptone reliable tests for indol are secured after relatively short periods of incubation. Bacto-Tryptone is also a very satisfactory nutriment for many bacteria.

PROTEOSE-PEPTONE

PROTEOSE-PEPTONE is a most useful ingredient of culture media designed for propagation of pathogenic bacteria. In media containing Proteose-Peptone the longevity of the organisms is increased and their virulence is maintained for long periods of time.

BACTO-BEEF EXTRACT

BACTO-BEEF EXTRACT is widely used in the preparation of culture media. It is completely soluble in the concentrations usually employed for culture media and has a reaction of pH 6.8.

BACTO-YEAST EXTRACT

BACTO-YEAST EXTRACT is rich in readily available forms of nitrogen. It is an excellent stimulator of bacterial growth and is particularly useful as an ingredient of culture media employed for propagation of fastidious types of organisms.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS

In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

DIFCO LABORATORIES

INCORPORATED

DETROIT 1, MICHIGAN

American Journal of Veterinary Research
COPYRIGHT 1946 BY AMERICAN VETERINARY MEDICAL ASSOCIATION
VOL. VII OCTOBER, 1946 NO. 25

Studies on Brucellosis in Swine. III. Brucella Infection in the Boar

L. M. HUTCHINGS, B.S., D.V.M., M.S., and F. N. ANDREWS, B.S., M.S., Ph. D.
Lafayette, Indiana

THE BOAR is commonly incriminated for a major part of the spread of brucellosis in swine. Reports over the past twenty-five years mention the importance of the boar both from the standpoint of susceptibility and transmission. One of the commonly accepted features of swine brucellosis is that the disease frequently affects the male. This situation is in contrast to that in bovine brucellosis, which is chiefly encountered in, and transmitted by, the female.

Connaway, Durant, and Newman[1] state: "The herd boar is susceptible and may contract the disease by association with infected sows—but whether by copulation or by ingestion has not been established." Hadley and Beach[2] showed that a boar experimentally infected with *Brucella suis* was able to transmit the disease to the sows he served shortly after receiving the inoculum. This boar showed a positive reaction to the agglutination test for brucellosis but overcame the infection within two months. These authors state: "The abortion bacilli, rarely, may be resident in the testicles of the boars, but since they have been isolated from infected testicles, it is proved that the boar may become a carrier of the disease." On the other hand, Hayes[3] was not able to produce infection by breeding infected boars to negative gilts in two cases, nor did he demonstrate localization of *Br. suis* in the testicles of 17 boars fed or injected with saline suspensions of Brucella.

Thomsen[4] reports: "In swine, the porcine Brucella infection is spread chiefly by the boars, and the infection is apt to take a particularly malignant course in the boar—which naturally gives a great deal of difficulty in breeding." He was able to show that 39 per cent of all the boars which he tested, in 61 infected herds of swine, were reacting to the agglutination test for brucellosis. Thomsen also states: "Autopsy and bacteriologic examinations were made on the genitals of 19 boars, 18 of which had given positive serum reactions for brucellosis before they were killed. Among these 19 boars, *Brucella suis* was demonstrated in one or several genital glands in 11. Often the presence of *Br. suis* was demonstrated in organs without any microscopic pathologic changes." In some experiments on transmission of infection by the boar, Thomsen was able to show that both of 2 boars were capable of transmitting the disease to 6 of 10 negative gilts. From these results, he concluded: "Transmission of the infection by the boar is a point of the very greatest significance; the sows are infected, in particular, through copulation." Concerning the Danish swine brucellosis epizoötic in 1929 and 1930, Thomsen states: "Spread of the infection through the covering boars was a conspicuous feature of the epizoötic. The

Published as Journal Paper No. 235 of the Purdue University Agricultural Experiment Station.

These studies were supported in part by a grant from the Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture.

most extensive infection transmitted by 1 boar involved 25 herds."

Cotton and Buck[5], in this country, found, upon inquiring into the nature of abortion in swine, that boars were more frequently infected than bulls, and that they probably played a much greater rôle in infecting sows than did the bull in infecting cows. Although several workers have been able to isolate *Br. suis* from various regions of the genital tract, and although there is considerable evidence to indicate that swine brucellosis can be transmitted by the boar, we have found no records of comprehensive studies of the actual elimination of *Br. suis* in the semen of the boar.

The primary objectives of the study reported here were to determine the frequency of elimination of *Br. suis* in the semen of naturally and artificially infected boars, and to correlate the bacteriologic findings of the semen with such features as agglutination response, blood and tissue cultural results, and clinical manifestation of brucellosis. During the past three years, studies have been conducted on a total of 30 boars as follows: 19 experimentally exposed weanling boars, 3 experimentally exposed sexually mature boars, and 8 naturally exposed boars, all of which became infected as weanling pigs. The studies undertaken consisted of applying the standard agglutination test to the blood serum of these boars, blood cultural examinations for *Br. suis*, collection and bacteriologic examination of semen for *Br. suis*, and bacteriologic examination of the testes and other organs of the boars which were either castrated or brought to autopsy.

EXPERIMENTAL PROCEDURE

The standard agglutination procedure, employing both the rapid and tube methods, was used in all agglutination tests conducted. The serum dilutions employed were 1 : 25; 1 : 50; 1 : 100; 1 : 200; and 1 : 400. Brucella antigens used were supplied by the United States Department of Agriculture, Bureau of Animal Industry, Beltsville, Md.

Blood cultural examinations were conducted, using the technique previously described.[6]

Semen collections were made by using an

TABLE I—Results of the Experimental Exposure of Weanling Boars to Brucella suis

Boar No.	Route of exposure	Agglutination response*	Blood cultural results†	Genital bacteriologic findings for *Brucella suis*‡	Time after exposure
925	Per os	Negative	—	Negative	3 mo.
959	Per os	Positive	+	Negative	8 mo.
932	Per os	Positive	+	Negative	5½ mo.
938	Per os	Positive	+	Negative	8 mo.
924	Subcutaneously	Positive	+	Positive testis	15 da.
950	Subcutaneously	Positive	+	Negative	8 mo.
958	Subcutaneously	Positive	+	Negative	8 mo.
931	Subcutaneously	Positive	+	Negative	5½ mo.
960	Per prepuce	Negative	—	Negative	8 mo.
933	Per prepuce	Negative	—	Negative	2 mo.
927	Per prepuce	Negative	—	Negative	8 mo.
936	Per rectum	Positive	+	Positive semen	5 mo.
949	Per rectum	Positive	+	Negative	8 mo.
930	Per rectum	Positive	+	Negative	8 mo.
941	Per conjunctiva	Negative	—	Negative	8 mo.
922	Per conjunctiva	Positive	+	Negative	24 mo.
929	Per conjunctiva	Negative	—	Negative	8 mo.
921	Intravenously	Positive	+	Negative	8 mo.
928	Intravenously	Positive	+	Positive seminal vesicles	5½ mo.

*Negative indicates no agglutination or incomplete agglutination at the 1:25 dilution. Positive indicates a complete agglutination at the 1:25 dilution or above. †—indicates *Br. suis* was not recovered from the blood. +Indicates *Br. suis* was recovered from the blood. ‡Bacteriologic results reported here include semen examinations of all boars but 924; complete autopsy examinations of boars 925, 932, 924, 931, 933, 930, 928, and testicular cultures only of the remaining boars.

artificial vagina and either a female in estrus, or a specially built dummy sow. The hair of the boar was clipped along the ventral abdominal wall and especially around the sheath. In most instances, the sheath of each boar was douched repeatedly with sterile physiologic saline solution prior to collection of the semen. The samples were obtained by using a separate artificial vagina for each collection. The equipment was cleaned after each collection by thorough washing with soap and water and soaking in 70 per cent ethyl alcohol. The semen was collected in sterile, wide-mouthed bottles of 4-oz. capacity.

Bacteriologic examinations of the semen, testes, and other tissues were made both by direct culture on tryptose-agar plates and by guinea pig inoculation. For the guinea pig inoculations with semen, it was necessary to inoculate large numbers of guinea pigs with each sample, or to centrifuge and wash the semen with physiologic saline two to three times prior to inoculation. Mortality of the inoculated guinea pigs was very high when the semen was injected without washing. The inoculated guinea pigs were destroyed and bacteriologic examinations made from the spleen and liver after approximately a four-week period of incubation.

Field observations had shown that some boars seemed to become infected at a very early age, even as young as 8 weeks. In view of these observations, 19 boar pigs were exposed to *Br. suis* cultures when 8 to 10 weeks of age. The exposures were made as follows: four *per os*, four subcutaneously, three *per* prepuce, three *per rectum*, three *per conjunctiva* and two intravenously. The detailed results of these exposures are shown in table 1.

It will be noted that infection, as measured by the agglutination response and recovery of *Br. suis* from the blood of these boars, occurred readily when exposures were made *per os*, subcutaneously, *per rectum*, or intravenously. The boar pigs exposed *per* prepuce and *per conjunctiva* failed to become infected with the exception of 1 boar pig (922) exposed *per conjunctiva*. Despite this rather favorable response to exposure, in which 13 of the 19 boar pigs apparently became infected, *Br. suis* was recovered from the testes of only 1 boar (924), and from the seminal vesicles of only 1 boar (928).

TABLE 2—Results of the Experimental Exposure of 3 Sexually Mature Boars to Brucella suis

Boar No.	Route of exposure	Agglutination response*	Blood cultural results†	Genital bacteriologic findings for *Brucella suis*‡	Time after exposure
539	Subcutaneous	Positive	+	Negative	12 months
410	Subcutaneous	Positive	+	Positive	6 months
109	Subcutaneous	Positive	+	Negative	12 months

*Positive indicates a complete agglutination at the 1:25 dilution or above. †+Indicates *Br. suis* was recovered from the blood. ‡Bacteriologic results reported here include only testicular examination at time of castration.

Subsequently, 3 sexually mature boars approximately 8 months of age were exposed subcutaneously to *Br. suis*. The results are shown in table 2.

All 3 of these boars became infected, as measured by both the agglutination test and blood cultural findings for *Br. suis*, and all developed an orchitis with visible enlargement of the testicle. *Br. suis* was isolated from the abscessed and fibrosed right testis of boar 410 at the time of castration six months after exposure. Upon castration of the other 2 boars, twelve months after exposure, both showed gross evidences of atrophy and fibrosis of one testis, but *Br. suis* was not recovered from either testis of either boar.

TABLE 3—Results of Studies with 2 Boars Naturally Exposed to Brucella suis

Boar No.	Agglutination response*	Genital bacteriologic findings for *Br. suis*†	
365	Positive	Positive	17 mo. after orchitis was noted.
952	Positive	Negative	17 mo. after orchitis was noted.

*Positive indicates complete agglutination at the 1:25 dilution or above. †Bacteriologic results reported here include two semen examinations of each boar and testicular cultures at time of castration.

Table 3 presents the detailed results of studies made on 2 boars (365 and 952) which were naturally infected as weanling pigs and developed a marked orchitis early in the disease. Two bacteriologic examinations of the semen for *Br. suis* of each of the boars were negative. Boar 365 was used to breed 2 gilts which were negative to the agglutination test, and both developed a low-titered agglutination response following breeding; *Br. suis* was re-

covered from the blood and colostrum of 1 gilt. These 2 boars were castrated seventeen months after the visible orchitis was observed. *Br. suis* was recovered from the right testis of boar 365. Boar 952 had a unilateral purulent epididymitis at the time of castration, but *Br. suis* was not isolated from either the testes or epididymides.

775, and 706) continuously eliminated *Br. suis* in the semen. Despite a steady decline in serum agglutination response in 5 of the 6 boars, a corresponding decline in the elimination of *Br. suis* in the semen was not observed.

These semen examinations covered a period from October, 1944, to April, 1945. Only 1

TABLE 4—Agglutination Response of 6 Naturally Infected Boars Before and During the Period of Semen Collection

Agglutination reactions at 1:25; 1:50; 1:100; 1:200; and 1:400 dilutions of serum.

Boar No.	9/6/44	11/9/44	12/28/44	3/15/45
660	+++++	++PPP	+++PT	++T−−
775	+++++	++P−−	PPTTT	++−−−
706	+++++	++PTT	TTT−−	−−−−−
704	+++++	++PT−	*+PT−−	++T−−
665	+++++	+++++	*++++P	+++++
708	+++PT	+PT−−	+PPT−	PT−−−

+Indicates positive agglutination; P indicates partial agglutination; T indicates trace of agglutination; −indicates no agglutination. *Unilateral castration performed 16 days prior to this test.

While conducting a field study in a Brucella-infected herd, it was observed that infection occurred in a high percentage of the weanling pigs. In 1943 and 1944, agglutination tests were conducted approximately four to six weeks after weaning on the spring pigs farrowed in this infected herd. In 1943, of 86 spring pigs tested, 48 (56%) reacted in various dilutions of the serum. In 1944, of 21 boar pigs tested, 13 were reactors, and of 58 gilt pigs tested, 16 were reactors. Six of the boar pigs which reacted to the agglutination test in 1944 were obtained for the detailed studies reported in tables 4 and 5.

Five of the 6 boars reacted completely at 1 : 400 dilution of the blood serum, and 1 boar (708) reacted incompletely at 1 : 400 dilution of the blood serum at the time they were tested as young pigs. During approximately six months of observation, there was a steady progressive decline in the serum agglutination responses of all but 1 boar (665). Two blood cultural examinations for *Br. suis* were negative on each of the 6 boars on Sept. 6 and Dec. 28, 1944.

The agglutination results with these 6 boars are given in table 4, and the results of bacteriologic examinations of the semen for *Br. suis* are shown in table 5. From the latter table, it may be noted that 63 (68%) of the semen examinations yielded *Br. suis* from a total of 92 examinations made. These results further show that all 6 boars eliminated *Br. suis* in the semen at some time during the observation period, and that 3 boars (660,

boar (704) was showing any visible evidence of orchitis or other clinical symptoms indicative of brucellosis when the studies began. Subsequently, boar 665 developed a slight, visible unilateral orchitis. Both boars showing orchitis (704 and 665) were castrated unilaterally, and *Br. suis* was recovered from the testis and epididymis of boar 704.

TABLE 5—Results of Bacteriologic Examinations for Brucella suis of the Semen of 6 Naturally Infected Boars

Boar No.	No. semen samples examined	No. semen samples yielding *Br. suis*	Per cent	No. samples negative for *Br. suis*	Per cent
660	18	17	94	1	6
775	17	15	88	2	12
706	16	15	94	1	6
704	10	5	50	5	50
665	15	3	20	12	80
708	16	8	50	8	50
Total	92	63	68	29	32

DISCUSSION

In this study which includes detailed observations on 30 boars, 22 experimentally exposed, and 8 naturally exposed, the susceptibility and importance of the boar in swine brucellosis is further emphasized.

Not only is the boar an important potential transmitting agent of the disease to other swine, but also a potential menace to other species of animals and to human health. The boar normally ejaculates between 125 and 500 cc.[7] of semen at one ejaculate, some of which is lost or spread about the premises during or following copulation. In two instances, direct culture of a single 5-mm. loopful of semen from a naturally infected boar yielded 800 to 1,000 colonies of *Br. suis* when streaked on a tryptose-agar plate, which indicates high concentration of *Br. suis* in such semen. These findings could well account for much spread of brucellosis in a swine herd from oral exposure, as well as coital exposure, to such semen. Furthermore, the handling of infected boars could well be a source of infection to the caretaker, veterinarian, and others having contact with Brucella-infected swine.

One of the outstanding features of this study was the complete lack of visible evidence of disease in 5 of the 8 naturally infected boars. Sex drive was relatively normal in 4 of 8 naturally infected boars and definitely subnormal in the remainder. In 2 of the boars (704 and 665), sex desire decreased to the point at which no semen collections could be made because of the failure of the boars to mount estrus sows.

Detailed physiologic studies of the semen of the 6 naturally infected boars were made, and the results are presented in a separate report[8]. Semen quality was definitely inferior to that reported for normal boars[9]. Semen volume was reduced and the length of survival of the sperm, when stored at 5 C., was shorter than normal. Sperm numbers were within the normal range in 5 boars (706, 708, 775, 660, and 665) and subnormal in the remaining 3. The results suggest that brucellosis may have a deleterious effect on both semen quality and sex desire.

Four of these boars (660, 706, 775, and 708) were used to breed a total of 25 brucellosis-free gilts, 2, 5, 8, and 10 gilts, respectively. None of the gilts developed positive agglutination reactions, nor was *Br. suis* recovered from their blood at any time following such breeding. These gilts farrowed litters which averaged below normal in size, as reported in detail in another paper[8] which appears on succeeding pages of this journal. These boars were from a naturally infected herd which had not been experiencing any considerable number of known abortions. These results fail to confirm the generally accepted concept that the infected boar is a primarily important means of spread of brucellosis to sows by coition.

It should be noted that the primary objective of this experiment was to study the elimination of *Br. suis* in the semen of brucellosis-infected boars. The study of the transmission of brucellosis by infected boars to normal sows, through coition, is in the exploratory stages at this station, and the data available at present should be regarded as of a preliminary nature, especially since the limited data reported here do not agree with vast clinical experience. The influences of copulation, artificial insemination, and ingestion *per os* of infected boar semen are being investigated in further experiments.

SUMMARY AND CONCLUSIONS

1) Boars, even young, weanling boar pigs, are apparently susceptible to brucellosis either by experimental or natural exposure.

2) Elimination of *Brucella suis* in the semen was a consistent finding in 6 of 8 naturally infected boars. In one series of 92 semen specimens examined from 6 boars, *Br. suis* was isolated 63 times.

3) Symptoms varying from a definite orchitis with decreased sexual desire to no visible symptoms were noted in naturally infected and experimentally exposed boars.

4) The intensity of the agglutination response was not a measure of the elimination of *Br. suis* in the semen of infected boars.

5) Thus, these studies indicate that the infected boar is commonly an important

reservoir of *Br. suis* and that the bacteria are freely disseminated in the semen. The potential menace of such boar semen to other animals and human health cannot be ignored.

References

[1]Connaway, J. W., Durant, A. J., and Newman, H. G.: Infectious Abortion in Swine. Univ. of Missouri Agric. Exper. Sta. Bull., 187, 1921.

[2]Hadley, F. B., and Beach, B. A.: An Experimental Study of Infectious Abortion in Swine. Univ. Wisconsin Agric. Exper. Sta., Res. Bull., 55, 1922.

[3]Hayes, Fred.: Some Studies in Swine Abortion. J.A.V.M.A., 60, (1922): 435.

[4]Thomsen, Axel.: Brucella Infection in Swine. Acta Path. et microbiol. Scandinav. Supplementum, 21, 1934. Translation by Hans Anderson, M. D.

[5]Cotton, W. E., and Buck, J. M.: Bureau of Animal Industry Researches on Infectious Abortion. J.A.V.M.A., 78, (1931): 306.

[6]Hutchings, L. M., Delez, A. L., and Donham, C. R.: Studies on Brucellosis of Swine. I. Infection Experiments with Weanling Pigs. Am. J. Vet. Res., 5, (1944) :195.

[7]Lambert, W. V., and McKenzie, F. F.: Artificial Insemination in Livestock Breeding. U. S. Dept. of Agric. Circular 567, 1940.

[8]Andrews, F. N., and Hutchings, L. M.: Studies on Brucellosis in Swine. IV. Semen Quality in Brucella-Infected Boars. Am. J. Vet. Res., 7, (1946): 385-387.

[9]McKenzie, F. F., Miller, J. C., and Bauguess, L. C.: The Reproductive Organs and Semen of the Boar. Univ. of Missouri Agric. Exper. Sta. Res. Bull., 279, 1938.

Research Fellowships Available

The Research Council of the American Veterinary Medical Association announces that a number of veterinary research fellowships are available for the academic year 1947-48.

Holders of fellowships must be veterinarians and must be citizens of the United States or Canada. Veterinary students who expect to graduate at the end of the current school year may apply for fellowships for the following year. The last date for filing applications for the year 1947-48 is March 1, 1947.

The Committee on Fellowships of the Research Council will meet early in April to consider applications and to award fellowships. Applicants will be notified of the action on their applications not later than May 15, in most cases earlier.

The stipend of the fellowships will be determined by the needs of the different fellows and usually will range from $100 to $150 per month.

For information concerning the fellowship project of the Research Council, refer to the February, 1946 (p. 107), issue of the JOURNAL of the AVMA.

It is planned to publish later a list of suggested research projects for the general information of applicants.

For application blanks and other information write:

Dr. H. H. Dukes, *Secretary*
A. V. M. A. Research Council
New York State Veterinary College
Cornell University
Ithaca, New York

Studies on Brucellosis in Swine. IV. Semen Quality in Brucella-Infected Boars

F. N. ANDREWS, B.S., M.S., Ph. D., and L. M. HUTCHINGS, B.S., D.V.M., M.S.

Lafayette, Indiana

NUMEROUS REPORTS during the past quarter of a century have indicated that swine brucellosis frequently affects the boar and is transmitted to other swine by infected males. Veterinarians and livestock producers have often observed breeding difficulties other than abortion in swine affected with brucellosis. Thomsen[1] reported that swine brucellosis "is apt to take a particularly malignant course in the boar—which naturally gives a great deal of difficulty in breeding". Hutchings[2] has previously reported that infection occurred in a high percentage of weanling pigs in a a naturally infected herd. Of 21 boar pigs tested, 13 were reactors, and of 58 young females, 16 were positive. Six of the male pigs from this herd were obtained for further study. A search of the literature has failed to reveal any detailed studies of the effects of swine brucellosis on the fertility of swine. No information has been found in the literature on the semen quality of Brucella-infected boars.

EXPERIMENTAL PROCEDURE

Six purebred Berkshire boars from the 1944 spring farrow in the above mentioned, naturally infected herd were used in this and other studies (Hutchings and Andrews[3]). All of the males showed high blood serum agglutination titers when tested by standard procedures[3], and *Brucella suis* was recovered from 63 of 92 separate semen collections. Semen samples were collected with individual artificial vaginas each Monday and Wednesday for a period of five weeks beginning on Oct. 25, 1944. The technique of semen collection is described by Hutchings and Andrews[3]. It was intended that physiologic observations would be made on ten consecutive semen collections from each boar. This was accomplished in all except boar 704, the only one showing evidence of orchitis at the beginning of the study. This animal had little sex desire and ejaculated only three times during the period in which physiologic studies of semen were being made.

The initial motility of the sperm was scored on a basis of 0 to 10 at the time of collection, at daily intervals thereafter on semen samples stored at 5 C., and at short intervals in samples incubated at 45 C.

A rating of 10 indicated that between 90 and 100 per cent of the sperm showed active progressive motility; a rating of 1 indicated that less than 10 per cent of the sperm were motile. The concentration of spermatozoa was determined by the method used for counting red blood cells with the standard hemacytometer. One dilution of each semen sample was made, and five separate counts of this diluted sample were made using five separate counting chambers. The volume of sperm cells was determined by the centrifugation of five semen-filled capillary tubes from each ejaculate; this technique is described by Shaffner and Andrews[5]. The following modification of the methylene blue reduction test, described by Beck and Salisbury[6] for estimating the quality of bull semen, was used: the methylene blue solution contained 50 mg. methylene blue, 4.76 Gm. sodium citrate, and distilled water to make 100 cc. One tenth cc. of methylene blue solution was added to 0.5 cc. of boar semen in a 5-cc. test tube. The semen was covered with a quarter of an inch of mineral oil, incubated in a water bath at 45 C., and the time required to reduce the blue color was recorded.

At the conclusion of the period during which physiologic and bacteriologic studies of the semen were being made, 4 of the boars (708, 775, 706, and 660) were naturally bred to 11, 10, 6, and 3 gilts, respectively. The experiment was not designed to measure semen quality by natural breeding results, and these data should be regarded as of a supplementary nature.

RESULTS AND DISCUSSION

The data on the semen characteristics of the Brucella-infected boars are summarized in table 1. With the exception of boar 704, from which only three samples

Published as Journal Series Paper No. 236 of the Purdue University Agricultural Experiment Station.

were studied, the mean values are of ten consecutive ejaculates.

The over-all semen quality of the 6 Brucella-infected boars was inferior to that reported for 7 normal, yearling Chester White boars by McKenzie, Miller, and Bauguess[4]. However, there were numerous differences in techniques and between the animals in the two studies, and thus only a few general comparisons can be made. Average semen volume in the infected boars was 69 ± 17.7 cc., in contrast with 262 cc. reported by McKenzie *et al.*[4] in normal boars. The average initial sperm motility of the infected boars was 7.4 ± 0.80. McKenzie, Miller, and Bauguess[4] scored motility on a 0 to 5 basis, rather than 0 to 10 as was used in these studies, and reported that initial sperm motility was, with a few exceptions, always 5. Although accurate comparisons between the two studies cannot be made, initial sperm motility appeared to be reduced in the Brucella-infected boars. McKenzie *et al.*[4] found a relationship between the time interval between collections and duration of sperm motility at 10 to 12 C. They reported that, in 5 normal boars, sperm remained motile for two and a half days when collections were made at twelve-hour intervals, three and a half days in samples collected at 24-hour intervals, four and six tenths days in samples collected at 48-hour intervals, and eight and a half days in samples collected at 72-hour intervals. In studies reported here, semen collections were never made at less than 48-hour intervals in the infected boars, and sperm were stored at 5 C. (a more favorable temperature for sperm survival than 10 to 12 C.). The average duration of motility of two and four tenths ± one and twenty-nine hundredths (2.4 ± 1.29) days is much less than that of four and six tenths (4.6) days reported in normal boars. The average concentration of sperm per cubic millimeter of semen was 161,000 in the infected boars, in contrast to 93,000 (range 15,000 to 480,000) in normal boars. However, due to reduced semen volume, the total number of sperm per ejaculate in the Brucella-infected boars (semen volume × concentration of sperm per mm.^{3}) was less than half that reported in normal boars (McKenzie *et al.*[4]).

TABLE 1—Semen Characteristics of Brucella-Infected Boars

Semen Characteristics	Boar Number						All
	660	706	775	708	704	665	
Av. semen volume (cc.)	47.3	76.1	65.7	86.4	58.2	79.8	69.4
Standard deviation	15.27	18.37	12.96	21.73	19.07	18.86	17.73
Av. initial motility	6.4	8.0	6.5	8.0	6.0	8.3	7.4
Standard deviation	0.88	0.78	1.0	0.61	1.0	0.59	0.80
Av. duration motility at 5C. (days)	1.3	3.4	1.6	3.4	1.5	2.7	2.4
Standard deviation	1.18	1.58	0.87	1.23	0.87	1.55	1.29
Av. duration motility at 45C. (min.)	39.1	57.2	47.6	54.8	34.7	45.4	48.4
Standard deviation	21.82	16.46	20.77	16.35	20.03	21.25	19.39
Av. methylene blue reduction time (min.)	11.5	8.4	12.0	9.7	9.0*	8.9	9.8
Standard deviation	3.00	6.17	9.49	6.78		2.64	6.42
Av. sperm concentration (1,000's/mm.^{3})	99.	266.	140.	199.	47.	112.	161.
Standard deviation	17.5	30.8	21.6	23.5	12.1	17.6	22.6
Av. sperm cell volume (%)	3.5	6.3	5.4	3.8	3.8	3.6	4.7
Standard deviation	0.34	0.68	1.33	0.45	0.45	0.54	0.62
Per cent of all samples positive for *Br. suis.*	94	94	88	50	45	20	

*1 sample only.

No data have been found in the literature regarding the survival of boar sperm at 45 C., methylene blue reduction time, and sperm cell volume.

Under field conditions, it was frequently observed that Brucella-infected boars suffering from unilateral orchitis have little or no sexual drive and may be, for all prac-

tical purposes, sterile. Boar 704 used in this study had visible unilateral orchitis. His sexual desire, always low, finally decreased to the point where collections of semen could not be made. As shown in table 1, the semen quality of this boar was below the average of the group in nearly all characteristics. Examination of the affected testis revealed gross abscess formation, fibrosis, and microscopic necrosis of both the germinal and interstitial elements.

The breeding records of 4 of the Brucella-infected boars (706, 708, 660, and 775) are summarized in table 2. Although these

TABLE 2—Breeding Records of Brucella-Infected Boars

Boar No.	No. gilts bred	No. gilts farrowed	Total pigs born	Av. pigs/ litter
706	6	5	35	7.00
708	11	10	55	5.50
660	3	2	11	5.50
775	10	8	39	4.87
Total	30	25	140	5.6

data are not considered adequate to measure the natural breeding efficiency of infected boars, they seem to offer further evidence that semen quality may be subnormal in boars affected with brucellosis. The average litter size of 25 Brucella-negative gilts which farrowed following service to these infected boars was 5.6 pigs and is in contrast with an average litter size of 7.69 pigs in 42 litters farrowed in 1943 by gilts of comparable age in the Veterinary Department experimental swine herd. In the Animal Husbandry Department experimental swine herd, Vestal[7] reported that the average litter size for 203 gilts which farrowed at 1 year of age was 8.19 pigs.

These data show a general relationship between semen quality as determined by several physiologic measurements and litter size. Boar 706, which sired five litters averaging 7 pigs each, was distinctly above average in all semen tests. The semen of boar 775, whose litters averaged 4.87 pigs each, was below average in all characteristics except sperm cell volume. All boars failed to settle at least 1 gilt, but since it is impossible to determine the breeding efficiency of an individual female during a particular estrual cycle, the failure of conception cannot necessarily be attributed to the boars If, as is suggested by these data, litter size was materially reduced in Brucella-infected swine as compared to normal swine, there are important economic implications.

SUMMARY

The average semen characteristics of 6 Brucella-infected boars were as follows: Semen volume, 69.4 ± 17.73 cc.; initial sperm motility, 7.4 ± 0.80; duration of sperm motility at 5 C., two and four tenths (2.4) ± one and twenty-nine hundredths (1.29) days; duration of sperm motility at 45 C., forty-eight and four tenths (48.4) ± nineteen and thirty-nine hundredths (19.39) minutes; methylene blue reduction time, nine and eight tenths (9.8) ± six and forty-two hundredths (6.42) minutes; sperm concentration per cubic millimeter, 161,000 ± 22,600; sperm cell volume, 4.7 ± 0.62 per cent.

The average size of 25 litters sired by 4 of the Brucella-infected boars was 5.6 pigs.

References

[1]Thomsen, A.: Brucella Infection in Swine. Acta Path. et microbiol. Scandinav., Supplementum, *21*, 1934. Translation by Hans Anderson, M.D.

[2]Hutchings, L. M.: Brucellosis in Swine. Proc. 47th Ann. Meeting of U.S. Livestock San. A., (1943): 52.

[3]Hutchings, L. M., and Andrews, F. N.: Studies on Brucellosis in Swine. III. Brucella Infection in the Boar. Am. J. Vet. Res., 7, (1946): 379-384.

[4]McKenzie, F. F., Miller, J. C., and Bauguess, L. C.: The Reproductive Organs and Semen of the Boar. Univ. of Missouri Agric. Exper. Sta. Res. Bull., *279*, 1938.

[5]Shaffner, C. S., and Andrews, F. N.: The Determination of the Concentration of Spermatozoa in Fowl and Bull Semen. Anat. Rec., *86*, (1943): 99.

[6]Beck, G. H., and Salisbury, G. W.: Rapid Methods for Estimating the Quality of Bull Semen. J. Dairy Science, *26*, (1943): 483.

[7]Vestal, C. M.: Feeding and Management Experiments with Brood Sows and Litters. Purdue Univ. Agric. Exper. Sta. Bull., *413*, 1936.

Brucellosis in Swine. V. Reproduction Studies with Naturally Infected Sows and Boars

L. M. HUTCHINGS, B.S., D.V.M., M.S., A. L. DELEZ, D.V.M., M.S., and C. R. DONHAM, D.V.M., M.S.

Lafayette, Indiana

DURING the spring and early summer of 1942, field studies were being conducted by Purdue University in several Brucella-infected herds of swine. One of these herds consisted of an assembled group of purebred Hampshires which was in the midst of a serious outbreak of abortions and concomitant sterility. Prevailing conditions were such that desirable studies could not be carried out on the farm; so 9 infected sows and 2 adult boars from this herd were presented to the Agricultural Experiment Station for study following this natural infection with *Brucella suis*. The 11 infected swine were high-priced individuals of excellent breeding and were selected by the owner as representative of his herd.

Previous history showed that 2 sows (815 and 813) had aborted in their second pregnancy. The other 7 sows had been bred repeatedly prior to their arrival at Purdue University, but only 4 were pregnant. Sow 800 died after farrowing in the fall of 1942; thus, this animal is not included in most of the recorded results.

These swine have been maintained under close observation for two years in an effort to determine the practicability of maintaining such high-priced, infected individuals and to evaluate their efficiency as breeding stock. During the observation period, the sows were kept as a unit separated from all other swine. These infected sows were bred repeatedly to farrow two litters per year which, if successful, would have resulted in 32 litters over the two-year period. During this period, 14 litters, or less than half the expected number, were produced. The boars used for service on these sows were satisfactory breeders when other sows and gilts were bred to them; hence, it is logical to attribute the failures in reproduction to defects in the reproductive abilities of the sows. Of the litters produced, only one was premature, and satisfactory materials for bacteriologic examinations were not available from this litter. The other 13 litters were born at full term and *Br. suis* was isolated from the colostrum of sow 808 only, of a total of ten colostral examinations. Negative bacteriologic results for *Br. suis* were obtained from representative pigs from 13 litters and also from the fetal membranes of eight litters examined. However, of a total of 129 pigs farrowed, 44 were stillborn and 17 were weak and died within two days after birth. The detailed results of the farrowings and bacteriologic examinations of these 8 sows are given in table 1.

Fig. 1—Cut surface of testis of boar R, showing fibrous tissue bands surrounding areas of apparent necrosis in the testis and several abscesses in the epididymis. Brucella suis was recovered from this organ.

Published as Journal Paper No. 240 of the Purdue University Agricultural Experiment Station.

These studies were supported in part by a grant from the Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture.

TABLE I—Farrowing and Cultural Results with 8 Naturally Brucella-Infected Sows

Sow No.	Farrowing date	Pigs born	No. dead	No. alive	Cultural results for *Br. suis* at parturition*
800	9/22/42	10	4	6	Negative
808	3/20/43	10	3	7	Colostrum positive
	10/13/43	6	0	6	Negative
	5/21/44	11	3	8	Negative
834	9/1/42	5	3	2	Negative
	4/26/43	9	3	6	Negative
	5/12/44	9	1	8	Negative
810	5/17/43	3	0	3	Negative
814	9/22/43	13	all died		Negative
	10/15/43	14	premature†		Not cultured
832	9/4/42	7	0	7	Negative
	5/25/43	11	2	9	Negative
827	7/24/43	14	all died		Negative
815	7/24/43	8	0	8	Negative
	5/21/44	9	1	8	Negative
813	Never conceived				
Totals		129	57	72	

*Bacteriologic examinations were made of pigs, membranes, and colostrum. †Breeding date not known; pigs appeared to be approximately three weeks premature.

Fig. 2—Section of testis of boar R. Portions of tubules showing extensive necrosis with marked proliferation and infiltration of the intertubular tissue. x 80.

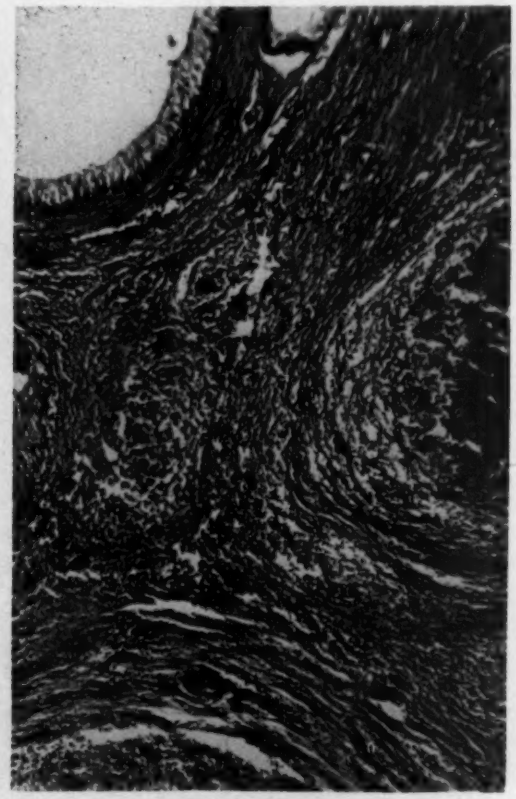


Fig. 3—Section of epididymis of boar R, showing a large area of cellular infiltration and necrosis of the intertubular tissue. x 80.

At no time during the observation period was *Br. suis* recovered from the blood of any of these sows or boars, indicating that the bacteremic stage of the disease had passed before the observations were made. A total of 28 blood cultural examinations were made at intervals of approximately six months. In this same period, the serum agglutination titers of these sows and boars receded from strongly positive levels to complete and/or incomplete reactions in the 1 : 25, 1 : 50, and 1 : 100 dilutions. The serum agglutination tests and blood cultural examinations were conducted according to previously described techniques[1]. The detailed results of the serum agglutination tests and blood cultural examinations conducted at irregular intervals are presented in table 2.

Limited data concerning the results of serum agglutination tests of pigs raised by 3 of these sows are shown in table 3. It will be noted that the pigs raised by 2 sows (810 and 834) were negative to the agglutination test at weaning and remained negative for two months. However, the pigs raised by sow 800 were showing low titer agglutination responses at weaning and, subsequent to weaning, this agglutination response diminished to completely negative reactions by the second month after weaning.

The 2 boars included in this study were showing clinical evidence of unilateral orchitis at the time they were obtained for observation. Their indisposition to breed prompted the owner to include both boars with the sows for study. Our efforts to breed any sows with these boars also met with little success. Consequently, in the spring of 1943, unilateral castration was performed on both boars to remove the affected testes to determine the effect on

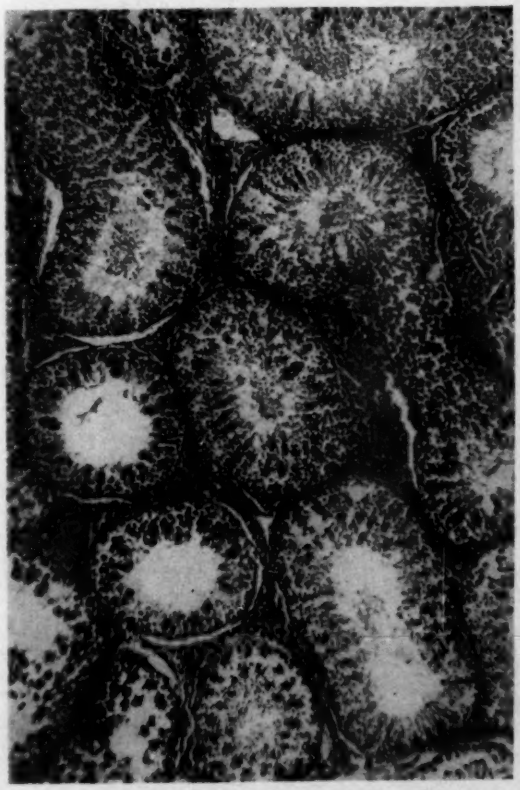


Fig. 4—Section of testis of boar S, showing apparent normal sperm formation. x 80.

Fig. 5—Section of portion of epididymis of boar S in which tubules are mostly empty. x 80.

TABLE 2—Results of Blood Agglutination Tests Conducted with Naturally Brucella-Infected Sows

Sow No.	Agglutination titers at indicated dates*										
	8/20/42	12/17/42	3/23/43	4/22/43	6/3/43	7/6/43	11/14/43	12/22/43	4/4/44	12/29/44	2/14/45
808	++P-- Neg.	++P--	+P--- Neg.	+P---			T---- Neg.		---- Neg.		
834	+++PT Neg.	+++--	+---- Neg.	PT---			PT--- Neg.			+PT--	
810	+++PT Neg.	+++PT	+PT-- Neg.	++P--	+++P-		+P--- Neg.			PT---	
814	PT--- Neg.	PPT--	P---- Neg.	PT---	++P--	PTT--	PPT-- Neg.			+PT--	
832	++P-- Neg.	++T--	+P--- Neg.	+P---		+PT--	PT--- Neg.		PT--- Neg.	PT---	
827	++PT- Neg.	++T--	+P--- Neg.	++T--	++PT-		+P--- Neg.			T----	
815	++++P Neg.		+PT-- Neg.	++++-	+P---		+PT-- Neg.		+PT-- Neg.	PT---	PP---
813	+++PT Neg.	+++PT	++PT- Neg.	++P--	+++P-		+P--- Neg.			+PTT-	
Boar S	+P--- Neg.							+P---	-----	-----	
Boar R			+++++	+++P-		++++P		++PT-	++P--	T----	

+=complete agglutination; P=partial agglutination; T=trace of agglutination; -=no agglutination; Neg.=*Br. suis* not recovered from blood.

*Both the standard plate and the tube agglutination tests were used. The 1:25, 1:50, 1:100, 1:200, and 1:400 dilutions were employed and the highest reaction reported regardless of the test used.

their breeding efficiency. *Br. suis* was recovered from the right testis of boar R. Bacteriologic examinations of the left testis of boar S were negative for *Br. suis*.

When the testis was removed from boar R, extensive adhesions were present. The testis was enlarged and firm with an uneven bulging surface as compared to normal testicular tissue. Gross examination of the cut surface (fig. 1) showed a marked alteration of the testicular tissue. The interlobular septa were considerably thickened and the tubules appeared as sharply rounded granular areas. The head of the epididymis showed multiple abscesses ranging from 3 to 18 mm. in diameter.

Histologic examination revealed that the normal structure of the testis had been rather completely altered (fig. 2). Large necrotic areas replaced most, if not all, of the areas normally occupied by seminiferous tubules. There was extensive proliferation of the intertubular tissue which was

TABLE 3—Blood Agglutination Titers of Pigs Born from Naturally Infected Sows

Sow No.	Pig No.	At weaning	After weaning	
			1 month	2 months
810	211	-----		
	212	-----		-----
834	123			-----
	124	-----		
	125	-----		-----
	126	-----		-----
	127	-----		-----
	129	-----		
834	16	-----		
	17	-----		
	18	-----		
	19	-----		
	20	-----		
834	102	-----	-----	-----
	110	-----	-----	-----
800	106	PT---	T----	-----
	111	PTT--	-----	-----
	101	PT---	-----	-----
	104	PPT--	-----	-----
	109	+PP--	-----	-----
	112	+PP--	-----	-----

Agglutination tests conducted at 1:25, 1:50, 1:100, 1:200, and 1:400 dilutions. + =complete agglutination. P=partial agglutination. T=trace of agglutination, - =negative agglutination.

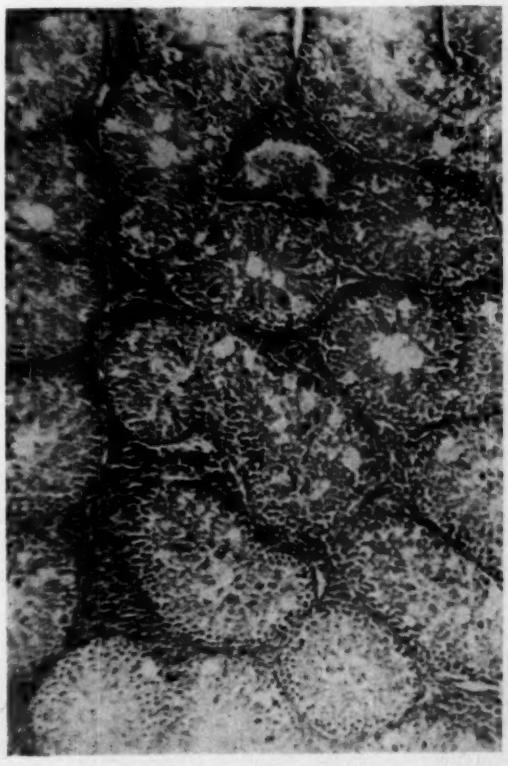


Fig. 6—Section of testis of normal boar. x 80.

Fig. 7—Section of portion of epididymis of normal boar showing numerous sperm cells in large tubules. x 80.

infiltrated with considerable numbers of lymphocytic and histiocytic cells. Many multinucleated cells of the Langhans' type were present. Portions of the epididymis showed extensive proliferation of the intertubular tissue with focal collections of lymphocytes and histiocytes, and areas of abscess formation and necrosis (fig. 3). Some areas of the epididymis observed in this case are similar to those found in such granulomatous infections as tuberculosis.

Boar S showed an enlargement of the left testis prior to its surgical removal. No other gross pathologic alterations of this testis were observed. Histologic preparations of the testis and epididymis did not show any evidence of damage to these structures (fig. 4 and 5), in spite of the definite visible enlargement of the gland. Some areas of the epididymis showed an apparent lack of sperm cells (fig. 5), but other sections showed tubules in which the sperm were plentiful. The testis of this boar was quite similar to those obtained from a normal boar (fig. 6 and 7).

Following recovery from the castration, these 2 boars were put into limited genital service with average results. The agglutination titer of boar R decreased from a complete reaction at the 1:1,600 dilution to incomplete at the 1:25 dilution within twenty-three months after unilateral castration. This boar had lost approximately 300 lb. in weight and commonly showed muscular tremors when aroused. Boar S never showed an agglutination reaction higher than incomplete at the 1:50 dilution of serum. This titer decreased to a completely negative reaction at the 1:25 dilution approximately one year after unilateral castration. Boar S did not lose much weight but did exhibit muscular tremors when aroused.

Following unilateral castration, both boars regained weight, the tremors ceased, and they returned to a quite effective breeding status. Boar S was used to breed 13 sows, 8 of which conceived and farrowed litters which averaged 11.25 pigs per litter. Boar R was used to breed 20 sows, 15 of which conceived and farrowed litters which averaged 9.26 pigs per litter.

DISCUSSION

From these results, it appeared that the continued maintenance of these Brucella-infected sows and boars could not be considered profitable except for the preservation of blood lines from exceptionally high-priced sows or boars. On the other hand, less detailed observations were made in naturally infected herds in which brucellosis apparently had existed over a period of years without serious economic losses.

These and other observations made on pigs raised from infected sows indicated that most pigs born from such sows do not contract the infection as suckling pigs; hence, there was good reason to believe that, under suitable conditions, the infected parent stock may be used as a nucleus from which to raise a brucellosis-free herd. Some litters, however, did develop an agglutination response while nursing which was apparently due to the passive transfer of agglutinins from the sow's milk to the blood serum of the pigs. In the case of sow 800, all 6 pigs were showing an agglutination response in low dilutions when they were weaned, but one month later these reactions had ceased. There are pigs which do become infected while nursing an infected sow. Mingle[2] and Thomsen[3], have evidence showing that entirely different results may be seen when the pigs nurse sows which are eliminating *Br. suis* in the milk. Isolations of *Br. suis* from the blood, as well as agglutination titers, were obtained from such pigs as long as twenty weeks after weaning. Thus, it can be seen that care must be exercised in interpreting the test results when weanling pigs are tested. In our experience, if the agglutination response in the weanling pig persists for more than one month after weaning, the pig should be considered as possibly infected.

The results of unilateral castration for the removal of an abnormal testis from Brucella-infected boars seems to merit fur-

ther study. Whether these 2 boars would be safe to use in a herd of brucellosis-free sows is not known, but such high-priced boars could be used to breed infected sows which would aid materially in maintaining desirable blood lines.

SUMMARY

1) Eight naturally Brucella-infected sows farrowed only 14 litters in a two-year period although they were bred repeatedly to farrow two litters per year.

2) Two Brucella-infected boars returned to an effective breeding status following unilateral castration for the removal of an enlarged testis, but evidence is not yet available to indicate that such boars could be used safely for breeding disease-free sows.

3) Pigs raised by these infected sows were either nonreactors at weaning time or became nonreactors within one month after weaning.

References

[1]Hutchings, L. M., Delez, A. L., and Donham, C. R.: Studies on Brucellosis of Swine. I. Infection Experiments with Weanling Pigs. Am. J. Vet. Res., *5*, (1944): 195-208.

[2]Mingle, C. K.: Personal communication.

[3]Thomsen, Axel: Brucella Infection in Swine. Acta Path. et microbiol. Scandinav. Supplementum, *21*, (1934). Translation by Hans Anderson, M. D.

Statement of Ownership and Management Required by the Acts of Congress of August 24, 1912, and March 3, 1933, for the American Journal of Veterinary Research. Pubished Quarterly at Chicago, Ill., as of Sept. 30, 1946: "State of Illinois, County of Cook. Before me, a notary public, in and for the state and county aforesaid, personally appeared Dr. J. G. Hardenbergh, who, having been duly sworn according to law, deposes and says that he is the managing editor of the American Journal of Veterinary Research and that the following is to the best of his knowledge and belief a true statement of the ownership and management of the aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, as amended by the Act of March 3, 1933, embodied in section 537, Postal Laws and Regulations, to-wit:

"(1) That the names and addresses of the publisher, editor, managing editor and business manager are:

Publisher—American Veterinary Medical Association, 600 S. Michigan Ave., Chicago 5, Ill.

Editor—L. A. Merillat, 600 S. Michigan Ave., Chicago 5, Ill.

Managing Editor—J. G. Hardenbergh, Chicago 5, Ill.

Business Manager—J. G. Hardenbergh.

"(2) That the owner is: American Veterinary Medical Association (a non-profit corporation), 600 S. Michigan Ave., Chicago, Ill. B. T. Simms, President, Bureau of Animal Industry, Washington, D. C.; J. V. Lacroix, Treasurer, Box 550, Evanston, Ill.; J. G. Hardenbergh, Executive Secretary, 600 S. Michigan Ave., Chicago 5, Ill.

"(3) That the known bondholders, mortgagees and other security holders holding 1 per cent or more of total amount of bonds, mortgages or other securities are: None.

(Signed) J. G. Hardenbergh."

"Sworn to and subscribed before me this Thirtieth day of September, 1946:
Helen S. Bayless, Notary Public (commission expires October, 1949)."

Comparative Growth of the Sheep and Bovine Animal During Prenatal Life

W. W. GREEN, Ph. D.

St. Paul, Minnesota

THE PRENATAL DEVELOPMENT of sheep and cattle has been studied sufficiently to permit a comparison of the intrauterine growth of the two species. As these species are somewhat related, and are in similar stages of development at the time of birth, some similarity in their actual or relative growth rates may be expected. Some differences should be indicated, however, by the unequal lengths of the gestation periods. A brief review of the various phases of the prenatal era will indicate some of the similarities and differences.

During the first week post-fertilization, the ova of the two species develop at the same rate (Green and Winters[1]). Divergence in the rate of development is soon noted, however, as the sheep ovum attaches to the endometrium about one day sooner (about 10 days) than does that of the bovine animal (about 11 days) or at the end of approximately 6.66 and 4 per cent of the respective gestation periods. Ova of both species are in similar stages of development at this time. The embryonic phase lasts about twenty-four days in the sheep and ten days longer in the bovine animal. At the beginning of the fetal period, both species are again in equivalent stages of differentiation (plate 1). Internally, all of the major organs and organ systems have been formed but will continue to grow and differentiate until later in the life of the individual. The fetal period continued about 116 days for the sheep and 234 days, or twice as long, in the bovine, or approximately 77 and 84 per cent of their respective gestation periods.

Because of the large differences between the species in terms of number of days of gestation or other absolute measures (*i.e.*, cm. or Gm.), it is desirable in many respects to compare the two species in relative terms; although it is always necessary to be mindful of both methods of measure at all times.

SOURCE OF DATA AND METHODS

Prenatal specimens of sheep and cattle of known ages have been recovered in this laboratory. Various bodily measurements made on fresh, unfixed fetuses have been published

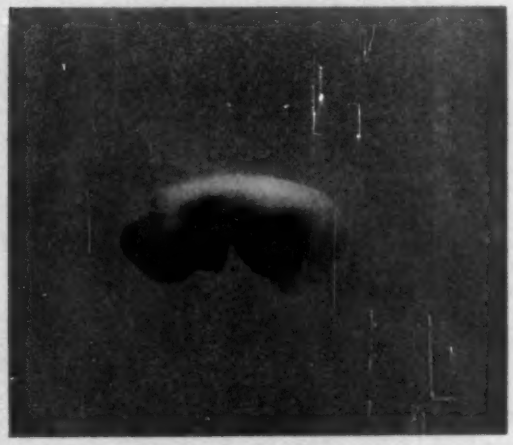


Plate 1—Forty-five-day, 15-hour-old bovine animal (the start of the fetal period.) x 1.

by Winters and Feuffel[2] and Winters, Green, and Comstock[3]. Those measurements, based on 3 to 7 specimens per age for the sheep and single specimens per age for the cattle, have been used for this study.

The age of the specimens has been transformed to the percentage of the gestation period by the formula: Age of fetus ÷ length of gestation (150 days, sheep; 280 days, cattle) × 100. For example, for a 70-day bovine fetus the formula would be 70 ÷ 280 × 100 = 25 per cent. In each species, the

Paper No. 2265, Scientific Journal Series of the Minnesota Agricultural Experiment Station. This work was supported in part by a Grant in Aid of Research of the Graduate School, University of Minnesota. The author is indebted to Miss Rita Firestone and Miss Jean Hirsch, director, Medical Art Shop, for assistance in constructing the graphs.

From the Division of Animal and Poultry Husbandry, University of Minnesota, University Farm, St. Paul.

last specimen or specimens were secured at the end of 93 per cent of the respective gestation periods.

Relative growth of bodily dimensions was calculated by setting the last group of data (that for the 140-day sheep and 260-day cow) equal to 100 per cent and figuring the measurements of younger specimens as a per cent of the last group of data. For example, the average weight of the 140-day sheep fetuses was 3,393 Gm., or 100 per cent. At 116 days, or 77 per cent of the gestation period, the average weight was 2,086 Gm. or 61.47 per cent of 3,393 Gm. The last bovine specimen was 260 days old and weighed 31,298 Gm. at the end of 93 per cent of its estimated gestation period. At 215 days, or 77 per cent of its estimated gestation period, a calf fetus weighed 16,102 Gm. When 31,298 Gm. was set equal to 100 per cent, the 215-day fetus weighed 51.45 per cent of the weight of the 260-day specimen. In those instances where graphs are used on actual rather than relative measures, this is indicated.

RESULTS AND DISCUSSION

The relative change in weight for each species and a comparison between the growth changes of the two species are shown in figures 1a and 1b. The arith-log graphing in figure 1b was used mainly to show the change and relationships during the earlier ages, which are not well-defined in figure 1a.

It will be noted here and elsewhere that the curve for the sheep data is of a more uniform character than that for the cattle. As previously mentioned, the points for the sheep curve were plotted from average figures secured from 3 to 7 specimens per age period while those of the cattle were based on but 1 fetus per age interval. The cows used for sources of bovine fetuses were of divergent genetic backgrounds. This, rather than inaccuracies in age determinations or

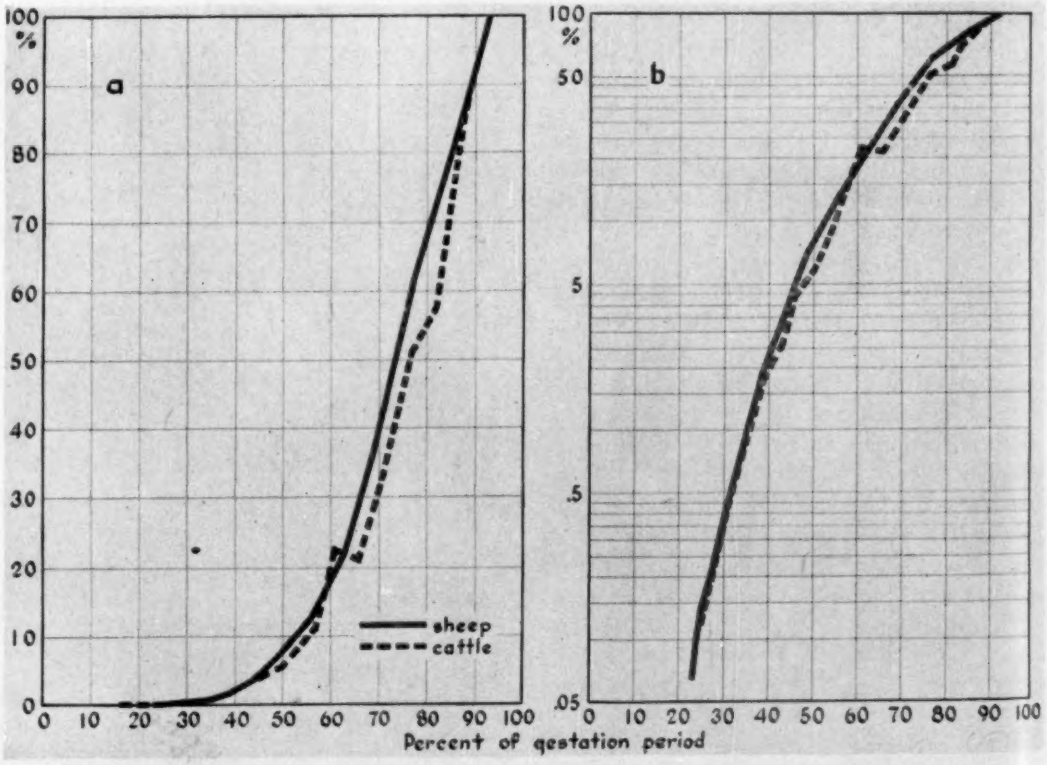


Figure 1—(a) Relative change in bodily weight of cattle and sheep fetuses in relation to the per cent of elapsed gestation period. (Weight in grams at 93% of the estimated gestation period was set to equal 100%, and interval weights were expressed as percentages of the final weight); (b) same data plotted on arith-log paper.

measurements, accounts for the irregularities. Some variations could have been eliminated from the cattle curves by discarding isolated portions of the data or by calculating regression curves from the data. Neither technique seemed justifiable. The irregularities do not detract from the main purpose of the graphs and they do indicate some of the variation that is to be found in individual cases. It is assumed that the use of greater numbers of specimens would smooth, but not alter the basic contour of, the curves in the case of both the sheep or the cattle.

In the case of figure 1b, as well as other similar graphs, no attempt has been made to calculate mathematical expressions of various apparent segments of the growth curves. The amount of data for either species is insufficient for accurate estimates. In addition, extreme care would have to be taken in such matters as the breeding, age, size, etc., of the parental animals and the closeness of the intervals of

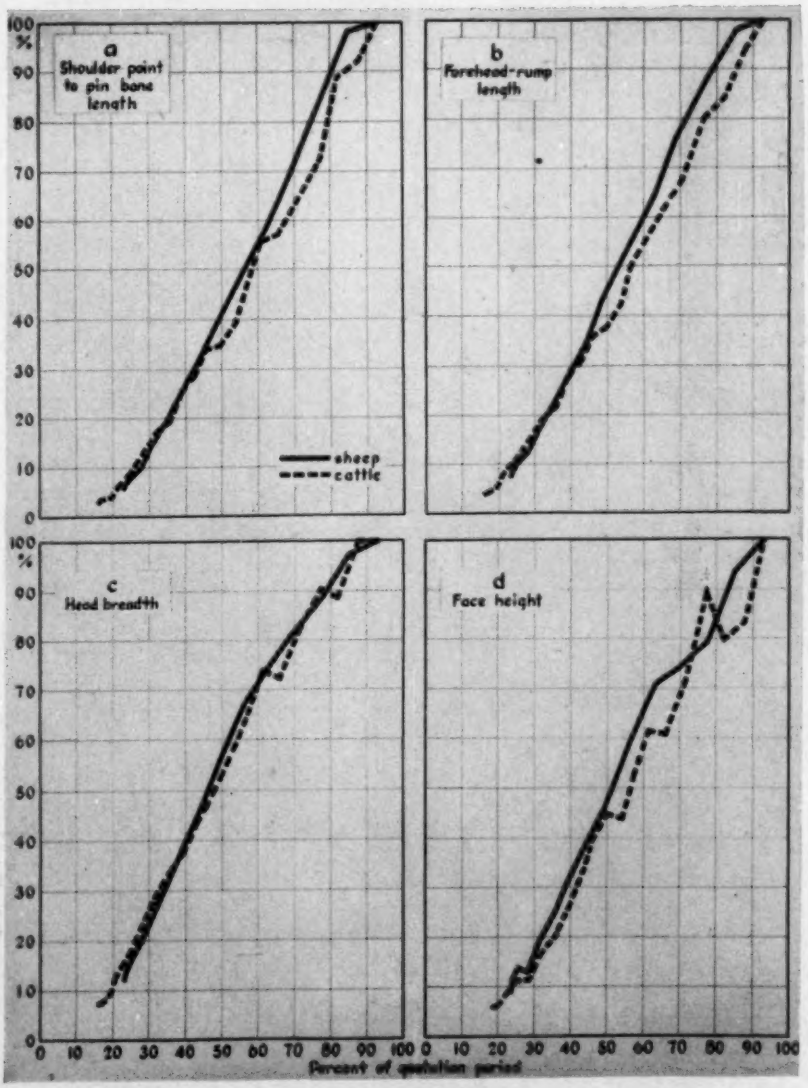


Figure 2—(a-d) Relative growth of indicated bodily parts compared to the elapsed per cent of the gestation period. See text for definition of measurements.

the fetuses; larger numbers of specimens would be needed before any reliance could be placed on any such calculations. Ponderal indices, etc., although calculated, are not to be presented for the same reasons.

Two measures of bodily length are shown in figures 2a and 2b. The shoulder point to pin bone length was secured by measuring the distance from the skin covering the anterior surface of the proximal end of the humerus, lateral tuberosity of the humerus in the adult, to the skin covering the posterior surface of the tuber ischii. This distance approximates the length of the body's trunk. The forehead-to-rump length was taken as the distance from the skin covering the anterior aspect of the forehead to the skin covering the posterior surface of the tuber ischii. This measurement in farm animals corresponds to the crown-to-rump length of humans. In both instances, the growth curves for the two length measures are similar for each species and maintain the same relative positions between species.

The head breadth, figure 2c, was measured at points just in front of the ears. The re-

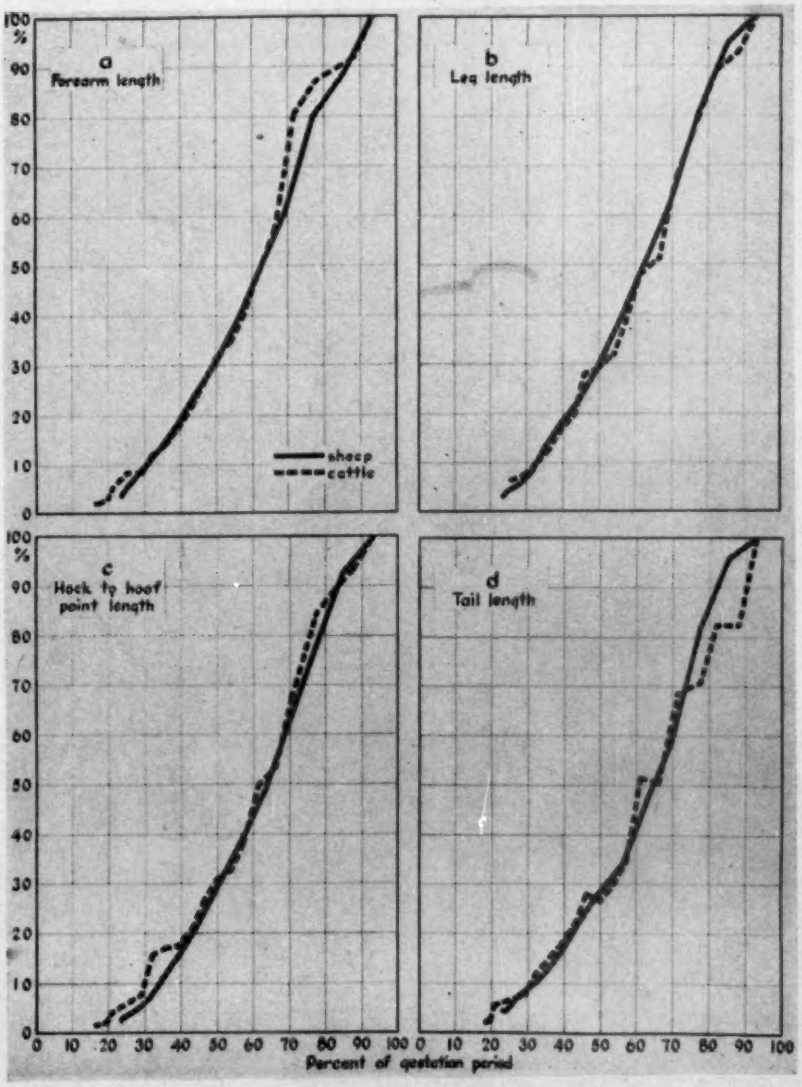


Figure 3—(a-d) Relative growth of indicated bodily parts compared to the per cent of the gestation period. See text for methods of measurement.

lative change for the sheep and cattle is almost identical. Less uniformity is shown in the growth of the height of the face, figure 2d. It was measured from a line joining the medial canthi of the eyes to the point of the chin.

One front leg, forearm (humerus), and two hind leg, leg length (tibia), and point of hock to point of hoof, measurements are shown in figures 3a, 3b, and 3c, respectively. These portions of the limbs, especially the two segments of the hind leg, grow in a similar pattern and the relative change is again alike in both species. Growth of the tail, in length, is shown in figure 3d. Circumferential measurements are illustrated in figures 4a, 4b, 4c, and 4d. The foreleg was evaluated by use of a string placed over the skin surface at the midpoint of the metacarpus. The circumference of the head was measured at a point above the eyes corresponding to the glabella

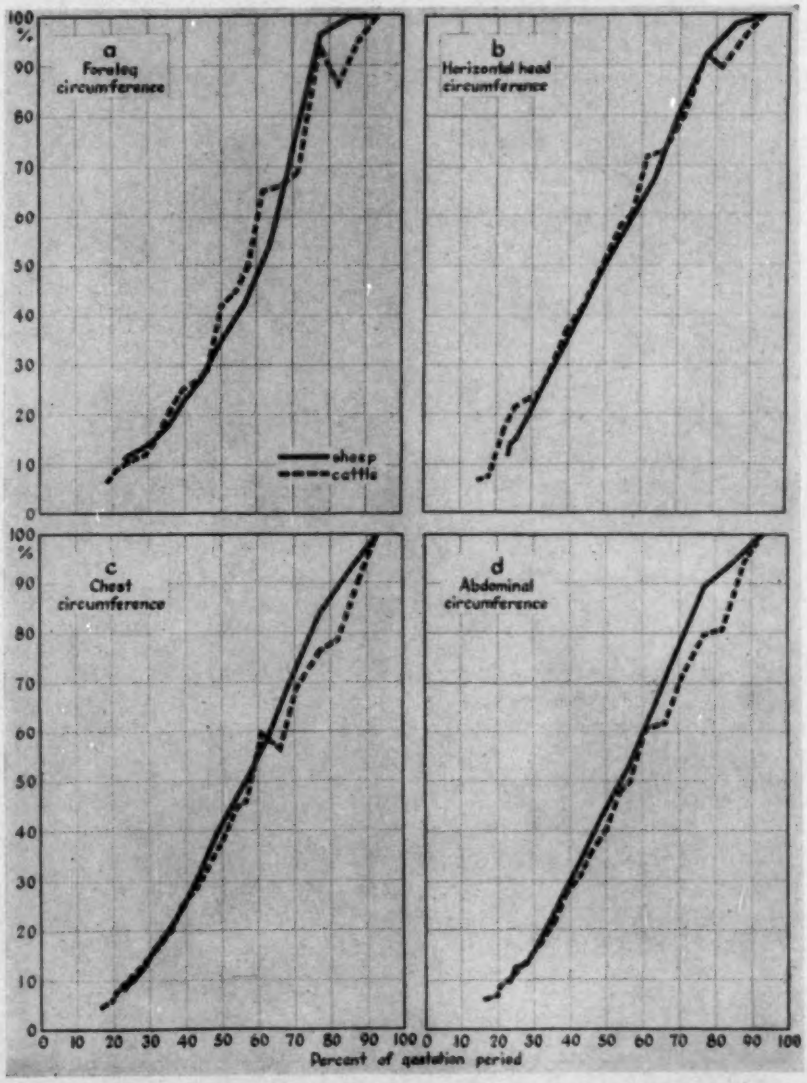


Figure 4—(a-d) Relative growth in circumference of the indicated bodily parts compared to the per cent of the elapsed gestation period. See text for definition of measurements.

in man. The chest was encircled just posterior to the front legs and the abdominal circumference was secured by passing a thread around the body just ahead of the attachment of the umbilical cord. The relative growth of the chest and abdomen was very similiar in both species. This parallelism is even more pronounced in figure 5a.

In all graphs of the next group, figures 5a to 5f, proportional growth of one bodily part is plotted against the proportional change of a second part. In figure 5a, a comparison of the growth changes of the chest and abdominal circumferences, it may be noted that the two grow at nearly the same relative rate in both cattle and sheep. A similar situation is found in figure 5c where the two measures of bodily length

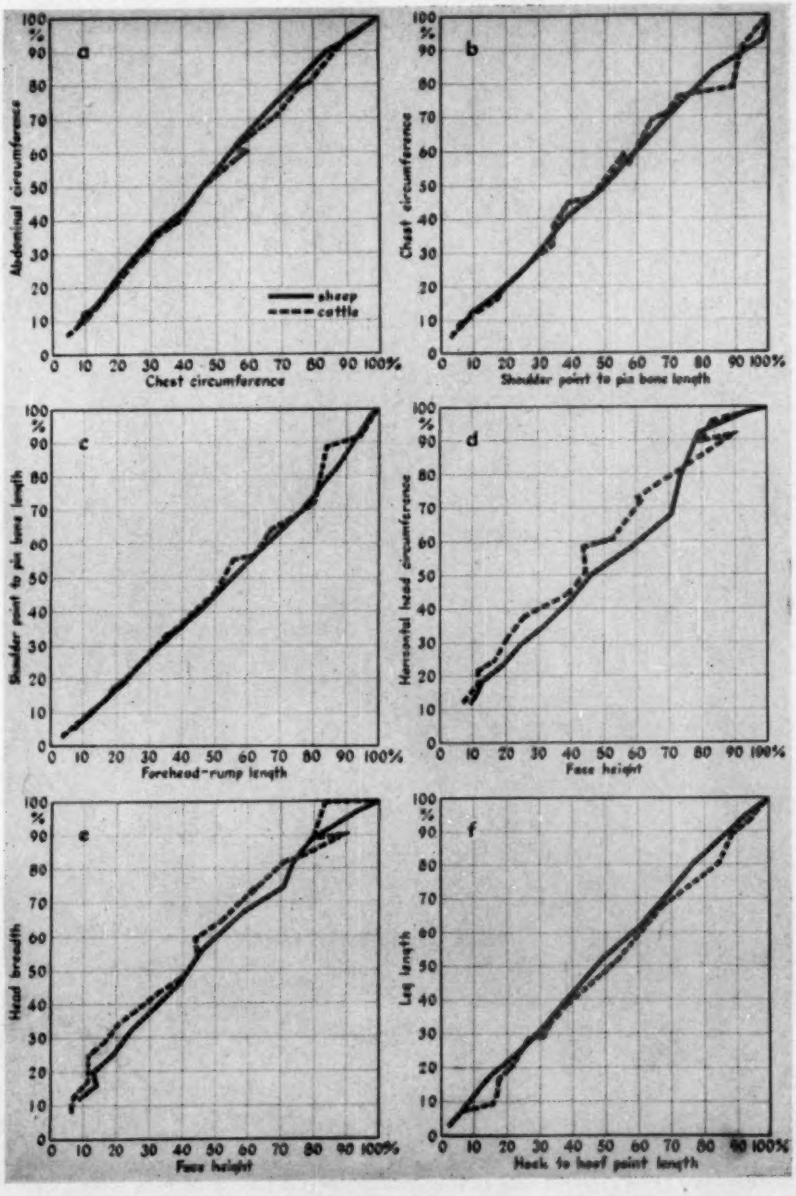


Figure 5—(a-f) Comparisons of the relative growth rates of the indicated bodily portions.

are compared. Because of the likenesses of the lines of figures 5a and 5c, all combinations of bodily length and circumferential measures are not shown; only the one of chest circumference and shoulder point to pin bone length, figure 5b.

The graphs comparing the horizontal head circumference, figure 5d, and head breadth, figure 5e, with the height of the face are approximate in their contours. This is not surprising as head breadth is a component of head circumference. Relative growth of two portions of the pelvic limb is shown in figure 5f.

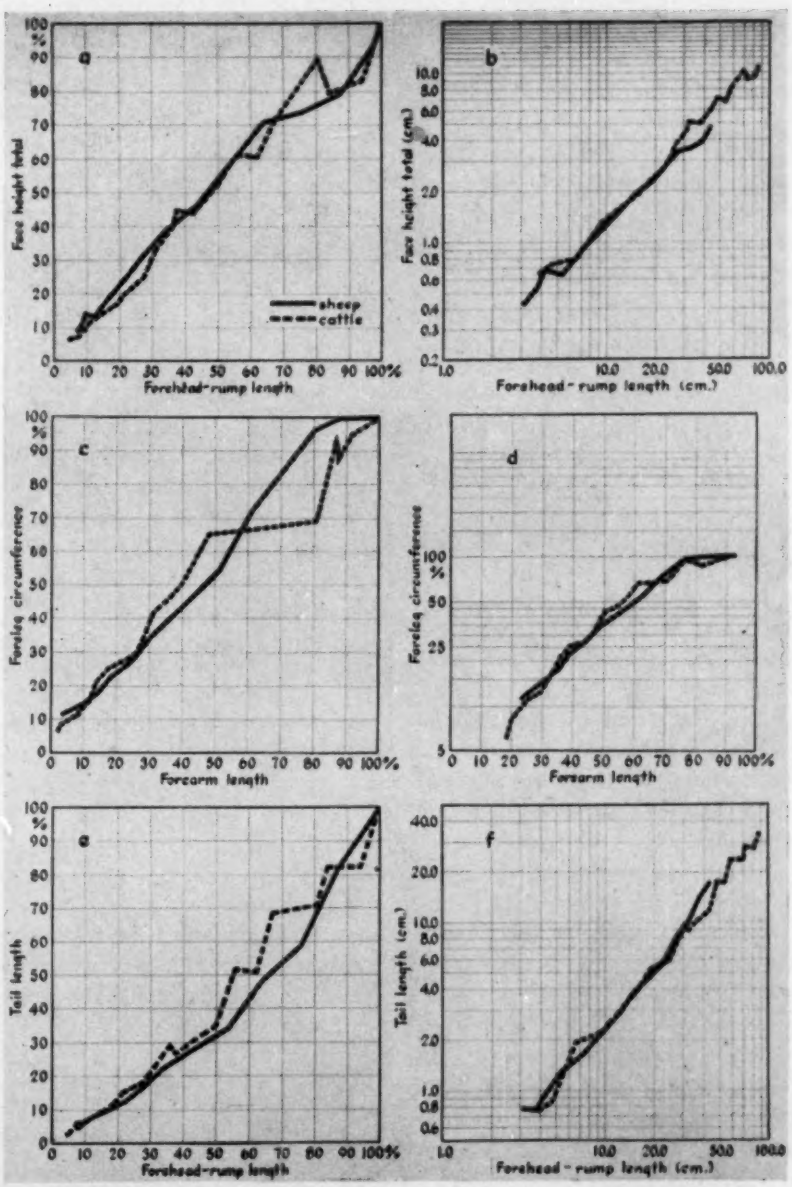


Figure 6—(a, c, and e) Comparisons of the relative growth rates of various bodily parts similar to figures 5a-f; (b and f) percentage growth rates as shown by log-log plotting of absolute measurements. (d) arith-log plotting of the same data as used in figure c.

Figures 6a, c, and e illustrate three additional comparisons of the same type as in figure 5. The same data used for figure 6c are shown in figure 6d, only plotted on arith-log paper. A different type of percentage growth rate may be seen in figures 6b and f where the original measurements used to calculate the charts in figures 6a and 6e have been drawn on log-log paper. It may be observed that, in the log-log graphs, the lines depicting the two species are in very close agreement. This suggests that, for the period of the prenatal existence of the sheep, certain bodily parts of the two species may grow at the same absolute rate. Further exploration of the data indicated this to be the case.

In many instances, the dimensions of the cattle and sheep were very similar at the beginning of their fetal periods. Likewise there is much agreement between some parts of the 140-day sheep and 160-day bovine specimens.

The difference in age between the 140-day sheep and the 160-day bovine animal is actually less than twenty days in terms of duration of fetal growth. As the sheep was 34 days of age at the start of the period and the bovine was 45 days old, the number of days spent as fetuses up to the age of comparison were 106 and 115 days, respectively.

Further study of the equality of growth was made by calculating linear regression coefficients of growth rates of the sheep from thirty-four to 140 days and cattle from forty-five to 160 days. Forehead-to-rump length was used as the independent variable for the determination of the coefficients, and the following dependent variables were used: chest circumference, shoulder point to pin bone, face height, and tail length. In no case did the regression coefficients of the cattle and sheep differ significantly. These results seem to indicate that the genetic and/or physiologic factors concerned in the two species are similar enough to allow approximately equal growth of some parts, at least, and that, for the duration of most of the fetal life of the sheep and during the same length of time in the bovine animal, the fetuses of the two species may, in some respects, be grouped into a common population.

SUMMARY AND CONCLUSIONS

The growth rates of some bodily dimensions of prenatal sheep and cattle have been studied and compared by use of relative and absolute measurements.

When fetal age was expressed in terms of the elapsed per cent of the gestation period and fetal measurements were expressed as per cent of final measures, the growth rates of the two species were very similar.

The data indicated that, up to a fetal age of 140 days in the sheep and 160 days in the bovine animal, some portions of the fetuses grew at approximately the same absolute rate and that, as far as some measurements were concerned, the fetuses of the two species could be grouped into a common population.

TABLE I—Dimensions of Cattle and Sheep Fetuses

Measurement	Unit	Start of fetal period bovine	Start of fetal period sheep	160-day bovine	140-day sheep
Weight	Gm.	2.77	2.20	3,562.00	3,393.00
Forehead-rump length	cm.	3.08	3.12	43.70	43.91
Shoulder point to pin bone	cm.	1.60	1.55	29.10	29.43
Chest circumference	cm.	3.20	2.68	32.00	33.60
Abdominal circumference	cm.	4.20	3.28	35.20	35.30
Foreleg circumference	cm.	0.60*	0.56	5.00	5.06

*This measurement was secured at 50 days of age instead of 45 days.

References

[1]Green, W. W., and Winters, L. M.: Prenatal Development of the Sheep. Minnesota Agric. Exper. Sta. Tech. Bull., 169, (1945): 36.

[2]Winters, L. M., and Feuffel, G.: Studies on the Physiology of Reproduction in the Sheep. IV. Fetal Development. Minnesota Agric. Exper. Sta. Tech. Bull., 118, (1936): 20.

[3]Winters, L. M., Green, W. W., and Comstock, R. E.: Prenatal Development of the Bovine. Minnesota Agric. Exper. Sta. Tech. Bull., 151, (1942): 50.

The Relation of Riboflavin to Equine Periodic Ophthalmia

MAJOR T. C. JONES, V.C., CAPTAIN THOMAS O. ROBY, V.C., and MAJOR FRED D. MAURER, V.C.

Fort Robinson, Nebraska

THE SIMILARITY of the ocular lesions in equine periodic ophthalmia to those in certain deficiency states, especially riboflavin deficiency, was outlined in a previous paper[1]. The present report contains additional experimental data concerning the relation of riboflavin to periodic ophthalmia. Horses were used in all of the experiments to be described. The accumulation of factual information about periodic ophthalmia has been an important result of our investigations. Clinical laboratory findings, especially concerning blood chemistry and the blood picture, are being reported separately[2].

TREATMENT OF ESTABLISHED CASES

The Acute Stage.—Since nutrition appeared to be involved in periodic ophthalmia, attempts were made to determine if any nutritional factors could be used in treatment. The effect upon acute cases was first explored, using crystalline riboflavin, brewer's yeast, and, later, ascorbic acid.

Experimental Procedure.—Riboflavin (U. S. P.) was mixed at the rate of 100 mg. to each 100 Gm. of brewer's yeast. Then 100 Gm. of this mixture was fed daily to each horse with acute periodic ophthalmia admitted to the hospital. Observations were made daily as long as the attack was acute. A total of 83 cases were treated in this manner but only the first 40 cases were considered in evaluating the results. The rest were all similar. Controls were selected from among cases with available clinical records. Each horse was matched with one receiving the riboflavin mixture on the basis of the severity of the symptoms at the outset and number of previous acute attacks.

The results in this experiment are summarized in table 1. It will be seen that 18 cases were of initial iridocyclitis and 14 were recurrent. Six cases of vascularizing keratitis, and 2 of catarrhal conjunctivitis were also included and matched with similar untreated cases as indicated above. It will be seen that the average duration of the acute symptoms was not significantly changed by the treatment nor did it have an appreciable effect upon the loss of vision following the attack. It is apparent that this method of treatment was of no value.

Experimental Procedure.—Six horses were treated by feeding riboflavin in brewer's yeast as indicated above and were also given 400 mg. of ascorbic acid intravenously on each of four days during the attack of acute iridocyclitis.

Table 2 portrays the results of this intravenous administration of ascorbic acid. It will be seen that the average duration of acute symptoms was eight and eight tenths (8.8) days as compared to an average of twenty-two and four tenths (22.4) to thirty-four and one tenth (34.1) days in the control group of table 1. Since the duration of acute symptoms is highly variable, no particular significance was attached to this finding when the work was completed. A study of the subsequent course of each of these cases revealed that only 1 out of the 6 had any recurrences. The great variation in the course of periodic ophthalmia makes it necessary to interpret these findings with caution, especially since so few horses were involved. However, the results are encouraging and suggest that this treatment should be explored further.

Subsequent Recurrences.—The effect of dietary supplementation upon the course of periodic ophthalmia was studied in several experiments. It was realized that the prevention of subsequent recurrences would greatly prolong the usefulness of many horses.

This work was conducted at the Veterinary Research Laboratory, Fort Robinson, Neb. The Laboratory was formerly located at Front Royal, Va.

Experimental Procedure.—In a preliminary trial, 20 horses with established cases of this disease were utilized. Ten of these animals were placed upon a high riboflavin diet and 10 upon a low riboflavin diet. During the first six months there were about one-fourth as many recurrences in the high riboflavin group as in the low riboflavin group. This effect seemed significant, but as the experiment progressed, acute attacks began to appear equally in each group. As a result, no clear-cut conclusion could be drawn.

A second experiment, started on March 1, 1944, was designed to explore the effect of several substances upon the course of periodic ophthalmia. The plan was to place an equal number of affected animals in each of two groups; one group to receive an ordinary ration; the other to receive the same basal ration, supplemented by other dietary factors. At first, riboflavin and niacin were to be added; then if no changes resulted, other factors were to be added singly.

At the start of this trial, 24 cases of periodic ophthalmia were available. They were divided into two groups with the cases paired as to severity of lesions. As cases were admitted to the hospital, alternate ones were added to each of the two groups, with the result that an equal number of animals were placed in each group by random selection. It was necessary to remove certain animals during this experiment due to complete blindness, or for use in other experiments, but this did not affect evaluation of the results obtained.

Feeding of these animals was divided into five different periods. During period 1, March 1 to May 17, 1944, the basal ration consisted of 15 lb. of timothy hay and 10 lb. of crushed oats. Group 1 (controls) received this ration alone. Each horse in group 2 (supplemented) received 50 mg. of riboflavin and 250 mg. of niacin each day in addition. All animals were fed in paddocks where they had free access to salt and water but no grass or other feed. During period 2, from May 18 to Sept. 11, 1944, 1 cc. of haliver oil was added to the daily ration of each horse in group 2. This oil contained 60,000 U.S.P. units of vitamin A and 600 U.S.P. units of vitamin D per gram. No other change was made in the ration during this period. Period 3 was from Sept. 12, 1944, to Feb. 27, 1945. The supplemented ration was changed to 15 lb. of timothy hay, 9 lb. of crushed oats, 0.5 lb. of dried skimmilk powder, 50 mg. of riboflavin, 250 mg. of niacin, and 1 cc. of haliver oil. The control ration remained the same. Period 4 was started on February 28 and ended March 13, 1945. During this time the same rations were fed as in period 3 but, in addition, 50 mg. of riboflavin and 250 mg. of niacin were administered subcutaneously twice weekly to each horse in group 2. All horses in this experiment became excessively fat; hence, during period 5, March 14 to May 15, 1945, the basal ration was reduced to 15 lb. timothy and 5 lb. crushed oats. Supplementation in group 2 remained the same.

TABLE I—Treatment of Acute Ocular Diseases by Feeding Riboflavin and Brewer's Yeast

Diagnosis	No. of cases		Average duration of acute symptoms		Cases with more than 25% loss of vision following attack	
	Treated	Control	Treated	Control	Treated	Control
Acute iridocyclitis (initial)	18	18	23.7	22.4	5	8
Acute iridocyclitis (recurrent)	14	14	36.5	34.1	10	9
Interstitial keratitis	6	6	24.8	26.0	1	0
Catarrhal conjunctivitis	2	2	41.5	17.5	0	0
Totals	40	40			16	17

General observations were made each day on all the horses in this experiment. Once a week, each horse was placed in a darkened chute and the eyes examined with a flashlight. The face of this flashlight was covered except for a small opening about 3.0 by 15.0 mm. which gave a narrow beam of light. The ophthalmoscope was used when further exam-

ination was indicated. A complete record was kept of all the ocular changes observed in these animals. Twenty-two horses were eventually included in the control group, and 19 in the supplemented group.

The effect of enriched rations upon the course of periodic ophthalmia is summarized in table 3. It will be seen from this table that 22 horses with an average of 1.68 acute attacks prior to the start of the experiment were used in group 1 which was fed an ordinary ration. Group 2 contained 19 horses which had suffered an average of 1.31 acute attacks prior to the experiment. This group was fed the enriched ration. The average number of days each horse was used in this experiment was 304.9 in group 1 and 387.3 in group 2. There were 32 recurrences in group 1 and 30 in group 2. The average number of horse-days for each acute attack was secured by adding the total number of days each horse was in the experiment and dividing this sum by the total number of acute recurrences. It will be seen that this gave a figure of 212.7 in group 1 as compared to 245.3 in group 2. The end result was measured by the number of horses in each group which suffered more than an estimated 25 per cent loss of vision. It will be seen that 59.0 per cent of group 1 were thus severely affected, compared to 47.6 per cent of group 2. Although the figures indicate a slightly better end result in the group receiving the enriched ration the difference is not considered significant.

The results of treatment of periodic ophthalmia by the methods described were generally discouraging with the possible exception of those following the use of ascorbic acid. It is our conclusion at present that treatment with riboflavin, niacin, vitamin A, or brewer's yeast is of doubtful value.

NORMAL HORSES ON LOW RIBOFLAVIN RATIONS

The effect of low riboflavin rations on normal animals was studied in several experiments. Numerous difficulties were encountered in the development of experimental rations for horses; not only was the choice of feedstuffs somewhat limited but many variable factors in their nutritional components demanded consideration. In these studies, the rations were changed frequently with only one factor remaining constant. The low riboflavin diet was always matched with a high riboflavin diet. The total caloric intake and proportion of protein, fat, and carbohydrate were varied. Generally speaking, most of the experimental rations were low in riboflavin and high in protein, the control rations being high in both protein and riboflavin.

Experimental Procedure.— Group 1 consisted of 9 horses which were maintained on a low riboflavin diet. Three yearlings, 3, 2-year-olds, 2, 3-year-olds, and 1, 7-year-old horse made up this group. Group 2 (control) was also made up of 9 horses, consisting of 3 yearlings, 3, 2-year-olds and 3, 7-year-olds. Group 1 was fed a low riboflavin diet from May 11, 1943, to July 10, 1945. The basal ration consisted of straw, dried beet pulp, oats, corn, linseed meal, and ground limestone; salt and water were available at all times. The control group received the same ration except that 80 mg. of riboflavin and 400 mg. of niacin were added daily for each horse.

In order to determine if sulfaguanidine could influence the intestinal activity of bacteria and thus the synthesis of riboflavin, this drug was administered to all these horses on two occasions. Starting on Jan. 30, 1945, 60 Gm. of sulfaguanidine was fed in the grain to each horse for three days. On three subsequent days, 30 Gm. were given to each horse. This treatment was repeated for six days, starting April 4, 1945. The results in these groups were essentially similar to those secured in groups 3 and 4 to be described later. For this reason, no further reference will be made to these animals.

The effect of low riboflavin rations upon normal mature horses was explored further. This experiment lasted about twenty-six months, from May 31, 1943, to July 10, 1945. Forty horses with no history of ocular disease were selected for this study. These animals, mostly geldings 8 years of age, were divided into two equal groups of 20 horses each. These groups were placed in adjoining paddocks and fed in open feed racks, group 3 receiving the deficient diet and group 4 the control diet. At the start, each horse was given an ophthalmologic examination. No le-

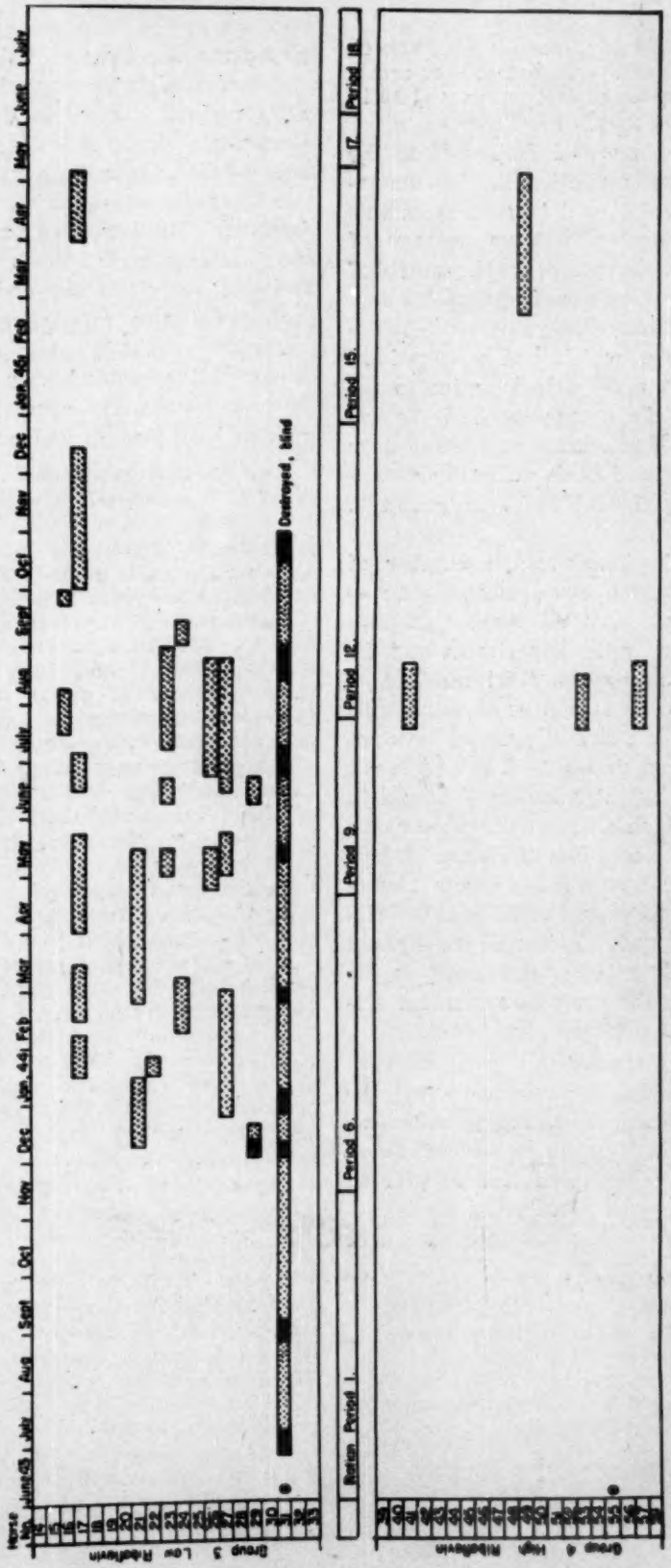


Chart I—Effect of Low Riboflavin Rations on Eyes of Normal Horses
Horse
Group 3 Low Riboflavin
Destroyed, blind
Ration Period 1.
Period 6.
Period 9.
Period 12.
Period 15.
17.
Period 18.
Group 4 High Riboflavin
Acute Iridocyclitis
Vascularizing Keratitis
Opacities in Vitreous at Outset

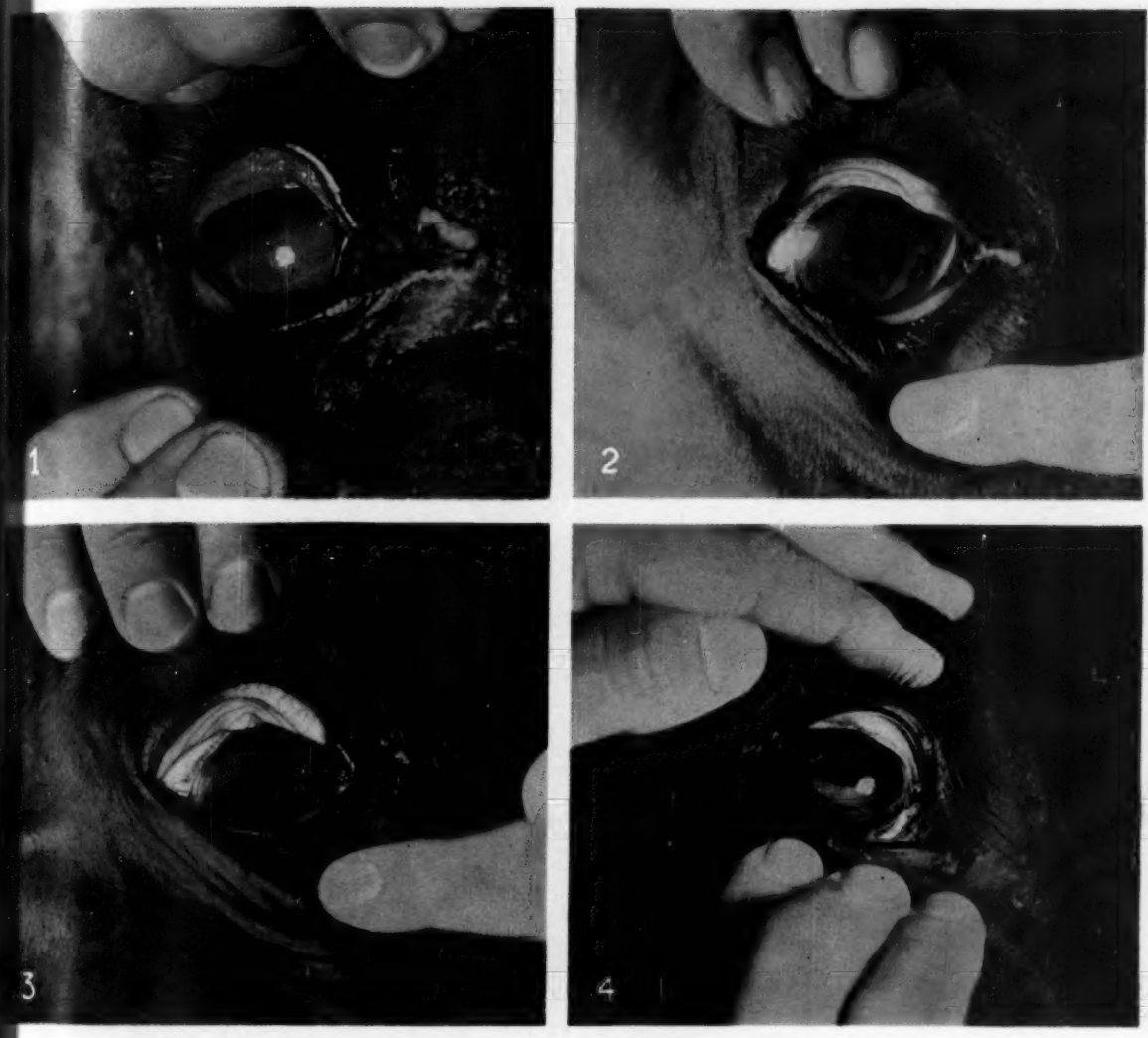


Fig. 1—Periodic ophthalmia, acute stage. Vascularization of the cornea.

Fig. 2—Periodic ophthalmia, acute stage. Myosis and exudate in the anterior chamber.

Fig. 3—Periodic ophthalmia, quiescent stage. The fluorescein test. The green dye appears in the aqueous of affected eyes following intravenous injection.

Fig. 4—Periodic ophthalmia, old case. Posterior synechia and cataract. The quartz-like lens is seen through the irregularly shaped pupil.

(The bright spot on the cornea in each of the photographs is a reflection of the light used and should be disregarded.)

sions of any kind were found in 38 of these animals. Two horses had punctate opacities in the vitreous humor of one eye. This lesion may indicate a prior attack of iridocyclitis but, since its significance is not always clear, 1 of these horses was left in each group.

ness. At the start of this experiment, the control group was fed natural substances high in riboflavin. When sufficient quantities of synthetic vitamins (at that time on government allocation) became available, the basal ration was made the same in both groups.

TABLE 2—Effect of Supplemental Feeding and Intravenous Administration of Ascorbic Acid upon the Acute Attack and Subsequent Recurrences

Horse no.	No. of prior acute attacks	Duration of acute symptoms (days)	Days observed subsequent to treatment	No. subsequent recurrences
Y18	2	4	335	3
Y28	1	4	365	0
Y81	3	24	365	0
9Y46	0	6	281	0
4Y95	0	9	265	0
8Y66	0	6	232	0
Average		8.8	307.1	

These animals, 31 and 55, will be discussed further when the results are considered.

Most of the horses in which periodic ophthalmia occurred at Front Royal were fat or even obese. It was desired to maintain the experimental animals in as good flesh as possible. For this reason, the quantity of feed was changed quite frequently depending upon the weather conditions and the appearance of the animals. More feed was required during cold weather. The riboflavin content of the ration fed to group 3 was always kept low, while that of group 4 was maintained at a high level. The major changes in these rations are shown in table 4. It will be seen that ground limestone was fed from July 21, 1944, to the end of the experiment. Before the addition of limestone, shifting lameness was observed in several horses of both groups, but after the calcium intake was raised there was no more of it. Acute indigestion, mild in nature, occurred infrequently. All animals survived the experiment except horse 31 which was destroyed due to complete blind-

Frequent analyses were made of these rations in order to determine their actual riboflavin content. The total riboflavin content of each ration is recorded in the last line of table 4.

The total digestible protein, digestible nutrients, dry matter, net energy, calcium, phosphorus, and potassium were also calculated for each experimental ration and considered before changes were made. Since these values are not especially important to this study, they will not be given. These figures can be calculated from the data given by using the average tables of Morrison[3]. All of the low riboflavin rations were high in protein, a factor which will be considered in the discussion.

The eyes of each horse were examined in a darkened chute once a week in the manner outlined above. If any new symptoms appeared at any other time during the week, the horse was caught and examined again in the darkened chute. All ocular lesions were recorded at the time of examination. Acute iridocyclitis was recognized by the presence of ptosis, photophobia, corneal vasculari-

TABLE 3—Summary of Effect of Enriched Rations upon the Course of Periodic Ophthalmia

Group	Total no. horses in group	Average no. acute attacks/ horse prior to start of experiment	Average no. days each horse was in experiment	Average no. horse-days per each acute attack*	Total no. recurrences	Horses with more than 25% loss of vision at end of experiment (no.)	Horses with more than 25% loss of vision at end of experiment (%)
Control (1)	22	1.68	304.9	212.7	32	13	59.0
Treated (2)	19	1.31	387.3	245.3	30	9	47.6

*This figure was secured for each group by adding the total number of days each horse was in the experiment and dividing this sum by the total number of acute recurrences in each group

TABLE 4—Effect of Low Riboflavin Rations on Normal Horses—Rations Used

Ration components	Period 1		Period 6		Period 9		Period 12		Period 15		Period 17		Period 18	
	Group 3	Group 4	Group 3	Group 4	Group 3	Group 4	Group 3	Group 4	Group 3	Group 4	Group 3	Group 4	Group 3	Group 4
Timothy hay, lb.	10	5									5	5	5	5
Wheat straw, lb.	5		10	10	10	10	9	9	4	4	7	7	9	9
Alfalfa hay, lb.		10												
Beet pulp, lb.									1	1				
Oats, lb.	5	5	9	9	9	9	9	9	7	7	7	7	9	9
Corn, lb.	3	3	2	2	2	2	2	2	2	2			2	2
Linseed meal, lb.			1	1	1	1			2	2	2	2		
Grd. limestone, Gm.							30	30	30	30	30	30	20	30
Riboflavin, mg.		40		50		80		80		80		80		80
Niacin, mg.						400		400		400		400		400
Haliver oil, cc.						1		1		1		1		1
Total Riboflavin, mg.	21.8	143.1	20.2	70.2	20.2	100.2	17.3	97.3	15.9	95.9	18.5	98.5	20.2	100.2

zation, epiphora, exudate in the anterior chamber, and myosis. Eyes with all the external symptoms (ptosis, photophobia, epiphora, corneal vascularization, and opacities), but without myosis or exudate in the anterior chamber, were also encountered. This condition will be designated "vascularizing keratitis" in this report. Its distinguishing feature is the appearance of many fine capillaries in the cornea. These may appear at any point on the circumference of the cornea but are usually most prominent at the dorsal limbus. Earlier changes can be recognized with the slit lamp and corneal microscope but the diagnosis in our cases was based upon naked-eye observation. A few cases of traumatic keratitis were encountered but will not be considered further.

The results of feeding low riboflavin rations to normal horses are portrayed in chart 1. This chart includes only those 40 animals in groups 3 and 4 which were described above. Attacks of acute iridocyclitis and vascularizing keratitis are recorded on this chart.

It will be seen that horse 31, which had vitreous opacities in one eye at the outset, had typical periodic ophthalmia within the first month. Nine attacks of acute iridocyclitis occurred in the succeeding months, the horse being destroyed in November, 1944. The development of the disease in this horse cannot be charged to experimental conditions. However, in the control group, horse 55, which started with similar opacities in the vitreous, had no other ocular disease during the experiment. Typical acute iridocyclitis appeared in horse 29 during the sixth month, but no recurrences were observed except for vascularizing keratitis in the same eye seven months later.

In the seventh month of the experiment (December, 1943), vascularizing keratitis began to appear in group 3 (low riboflavin). It will also be noted that this condition had a tendency to recur. The close similarity of vascularizing keratitis and periodic ophthalmia encouraged us to believe that the low riboflavin diet might be responsible for the incidence of periodic ophthalmia, since during the first year of the experiment vascularizing keratitis was not seen in group 4 (high riboflavin). Dur-

ing the second year, however, 3 cases of vascularizing keratitis did develop in the control group. Furthermore, the disease did not continue to progress in affected animals, nor did more cases develop in the low riboflavin group.

It is apparent from this experiment that riboflavin is not the only factor involved in periodic ophthalmia, although it does appear to be important. Optimum conditions favoring the development of periodic ophthalmia were not attained although they apparently were nearly reached during the first year.

DISTRIBUTION OF RIBOFLAVIN IN THE EQUINE BODY

The distribution of riboflavin and other nutritive substances in the equine body is of interest. It is known, for example, that riboflavin and niacin each have definite rôles in tissue metabolism[4] and their concentration is one clue to their activity in a particular organ or tissue. Analyses were performed upon certain equine tissues for the purpose of securing as much information as possible.

Experimental Procedure.—Equine tissues were collected promptly after destruction of the animal by shooting. Each tissue or organ was placed in a suitable container and stored in a dry ice cabinet at –70 C., until enough specimens were available for analysis. Ocular tissues and fluids (corneal epithelium, corneal stroma, lens, aqueous, vitreous, and uvea) were dissected out and each stored separately. Riboflavin was determined by the fluorometric method of Conner and Straub[5], or the microbiologic methods of Snell and Strong[6] or Landy and Dicken[7], the last being used most extensively for this determination as well as when other components of the B complex were assayed.

When sufficient tissue was available, each organ was analyzed separately. When certain ocular fluids and tissues were tested, it was often necessary to pool tissues from several horses. All samples were collected at the same hour in the morning before the horses were fed. Records were kept of the ocular history and conditions in each animal at the time of death as well as the exact ration components whenever indicated. In some cases, the vitamin content was determined upon the wet specimen; then the moisture content was found and the vitamin content calculated on the basis of dry weight.

TABLE 5—Riboflavin in Tissues and Fluids of Horses Fed Ordinary Rations

Number of analyses	Ocular condition	Aqueous	Vitreous	Adrenals	Liver
20	Affected eyes	0.31	0.17	8.23	28.35
20	Normal eyes	0.27	0.19	8.49	29.00

The Conner and Straub fluorometric method was used in these determinations.

It will be seen from table 5 that riboflavin is present in slightly higher amounts in the tissues of horses with normal eyes than in others affected with periodic ophthalmia. In these animals, all fed the same ordinary ration, this difference was neither great nor highly significant.

Further studies on the distribution of riboflavin and niacin in equine tissues are outlined in table 6 where several facts of interest are disclosed. It will be seen that the corneal epithelium is especially high in riboflavin and niacin, a feature that underlines the importance of these vitamins to this structure. It will also be seen that both niacin and riboflavin are present in somewhat greater amounts in most tissues of normal horses; this despite the fact that all of these animals were fed the same ration.

The effect of the riboflavin content of the ration upon the amount of this vitamin in the tissues is outlined in table 7. The concentration is greater in the tissues of those horses fed a high riboflavin diet; however, the disparity is not great, even in those animals which had been maintained upon their respectiive rations for more than a year. Of particular interest is the greater amount of riboflavin found in the contents of the large colon as compared to

that of the feed, this difference being greatest in those animals fed the low riboflavin ration. This is presumptive evidence that bacterial synthesis of riboflavin occurs in the equine intestinal tract.

Factors influencing bacterial synthesis of riboflavin in the digestive tract are of importance but are not thoroughly understood. For example, the effect of the high protein rations used in these experiments (*see*, table 4) will require additional study.

TABLE 6—Riboflavin and Niacin in Equine Tissues and Fluids (Horses on Ordinary Rations)

Ocular conditions	No. of eyes represented	Micrograms riboflavin per gram						
		Corneal epithelium	Corneal stroma	Lens	Aqueous	Vitreous	Uvea	Lacrimal gland
Affected eyes	40	13.94	1.08	1.19	0.63	0.47	1.97	7.6
Normal eyes	40	15.13	1.21	1.63	0.48	0.61	1.53	7.6
		Micrograms niacin per gram						
Affected eyes	40	119.5	47.8	30.3	3.03	2.64	29.0	46.9
Normal eyes	20	165.8	74.8	38.5	2.30	2.58	30.8	48.3

The Landy-Dicken microbiological method was used in all these determinations. This method usually gives values slightly higher than fluorometric methods.

OCCURRENCE OF RIBOFAVIN IN FEEDSTUFFS

At the time our interest first became focused upon the relation of nutrition to periodic ophthalmia, we decided to determine what effect feeding practices or quality of forage could have upon the quantity of riboflavin in the ration. The first step was to analyse horse feeds from regions where periodic ophthalmia was prevalent and also from other regions where it rarely occurred. Army stations were selected because their feeding practices were known, and records were available as to the incidence of periodic ophthalmia. Samples of feeds were collected from Fort Robinson, Neb., Fort Sill, Okla., and Fort Reno, Okla., where the incidence of periodic ophthalmia was low. Other samples were collected from Front Royal, Va., and Fort Bragg, N. Car., where incidence was much higher.

Wide disparity in the riboflavin content of these feeds was found. The riboflavin content was sometimes, but not always, related to the obvious quality of the forage. Many of the samples from stations with a high incidence of periodic ophthalmia were low in riboflavin. This was not always the case and no clear-cut correlation could be demonstrated between the distribution of riboflavin in the feed and the incidence of periodic ophthalmia.

When these data were studied in another way, it was clearly demonstrated that great variation in riboflavin intake can occur. Generally speaking, the tendency is to feed less hay and more concentrates where hay is more expensive or of poorer quality. Thus, we find that, in the western arid or semiarid regions, where heavy rainfall does not occur to interfere with hay production, hay is generally of better quality, cheaper, and hence comprises a greater part of the ration. In the eastern states, where rainfall is greater, hay production is more difficult, and hay is often poorer in quality and more expensive; therefore, concentrates usually form a greater part of the equine ration. Since riboflavin is both water soluble and destroyed by sunlight, hay that has been rained upon and bleached is lower in this vitamin.

The incidence of periodic ophthalmia is generally higher in areas of greater rainfall and much lower in arid or semiarid regions[8]. At the Front Royal Remount Depot, rainfall is greater and the incidence

of periodic ophthalmia much higher than at Fort Robinson, Neb. The feeding practices at the Front Royal Remount Depot are generally similar to those followed by civilian horsemen in the eastern part of the United States. Hay is fed in controlled amounts and concentrates are used liberally. Feeding of horses at the Fort Robinson Remount Depot is more nearly like that of civilian-owned animals in the west. Hay is supplied very liberally and grain in a more limited manner.

To illustrate possible disparities in quantity of riboflavin available in horse rations, two extreme examples shall be cited. A typical maintenance ration at Fort Robinson consists of grass or prairie hay fed at will with a somewhat restricted allowance of grain. Using high figures (based upon actual analyses of feed from Fort Robinson) an estimate can be made of the riboflavin content of this ration. For convenience, the riboflavin content is expressed in milligrams per pound of feed. These values were reported in micrograms per gram in a previous publication[1].

Fort Robinson ration	Amt. lb.	Riboflavin mg. per lb.	Total riboflavin mg.
Grass hay	18	2.50	45.00
Oats	6	0.97	5.82
Corn	2	1.27	2.54
Totals	26 lb.		53.36 mg.

In contrast, a ration fed at Front Royal shall be examined. At first glance, this appears to be a much better ration than described above. Using low figures (based upon actual assay of feeds at Front Royal) the estimated riboflavin content is quite different:

Front Royal ration	Amt. lb.	Riboflavin mg. per lb.	Total riboflavin mg.
Timothy hay	10	0.55	5.50
Alfalfa hay	5	1.34	6.70
Oats	9	0.42	3.78
Corn	2	0.52	1.04
Totals	26 lb.		17.02 mg.

Although these are extreme examples and most rations fall somewhere between them in riboflavin content, it is apparent that great variation in total riboflavin can occur. This is influenced both by the vitamin content of the feeds available and by the feeding practices in vogue. Actually, we have found rations which, by analysis, approached both these extreme examples. Other nutritive elements might be varied in much the same manner as illustrated in the above examples. Such additional factors are being studied for future report.

The nature of pasture herbage is subject to great variation and is another factor affecting the riboflavin intake; however, it cannot be fully considered here. Generally speaking, the better types of soils will support pastures containing more legume plants which are higher in riboflavin than most grasses. The nature of grazing land must be considered, but not enough data are available to justify any conclusions in this regard.

PREVENTION OF PERIODIC OPHTHALMIA WITH RIBOFLAVIN

The high incidence of periodic ophthalmia at the Front Royal depot resulted in unexcelled opportunities for study of control measures. Since 1939, all affected animals were under the direct control of the officer-in-charge of the Veterinary Research Laboratory. For several years, all new cases of the disease had been isolated, but although this made it possible to study the clinical course of many cases, segregation had no effect upon the incidence of periodic ophthalmia.

The feeding of synthetic riboflavin was started as soon as sufficient quantities of the chemical became available.

Experimental Procedure.—A group of horses was selected to receive crystalline riboflavin in their feed. It was deemed essential that all horses in the group should have a high past incidence of periodic ophthalmia and should be available for continuing study. One lot, consisting of about 130 horses, had been particularly plagued by periodic ophthalmia for some time. These animals were the officers' private mounts being maintained at the depot while their owners were overseas. The annual new case rate during 1943 had

been 109.56 per thousand. This was particularly high when compared to the rate for similar groups at the depot which was 50.14 per thousand during this same year. These horses were kept in one group and fed, for the most part, in open feed racks. A few of the older, less thrifty, or timid animals which did not thrive well in a large group were kept in an adjoining paddock and fed individually in the stable. All of these horses received the same ration at the hands of the same attendants. There was very little grass available in the paddocks where these horses were kept. During most of 1943, the ration for these horses consisted of: timothy hay 15 lb., oats 9 lb., yellow corn 2 lb., and bran 1 lb. Part of the time, 5 lb. of alfalfa was fed in place of an equal amount of timothy. During 1944 no alfalfa was fed.

TABLE 7—Riboflavin in Tissues and Fluids of Affected Horses Fed Low and High Riboflavin Rations

Type of ration	No. of horses	Riboflavin content in micrograms per gram						
		Total ration	Large colon contents	Adrenals	Liver	Kidney	Aqueous	Vitreous
Low riboflavin	8	2.20	10.96	7.57	27.70	18.20	0.55	0.58
High riboflavin	8	11.93	14.03	9.12	29.71	21.00	0.60	0.64

*Total ration and colon contents determinations are calculated on a dry weight basis. At least two specimens of each tissue were assayed from each horse. The fluorometric method of Conner and Straub was used in these studies. The horses in this experiment were maintained upon their respective rations for at least a year prior to their destruction.

Sick animals were detected by trained attendants and brought to the Veterinary Station Hospital for diagnosis and treatment. Any horse showing eye symptoms was also brought to the hospital. A veterinary officer also rode through these animals in the paddock and observed them at least once each week. This same system for the detection of sick or injured animals was followed with all the pasture-fed animals at the depot.

Beginning Nov. 12, 1943, crystalline riboflavin was added to the ration of these horses. No other change was made in their feeding or management. They were kept in the same paddocks and stable and cared for by the same personnel. No changes were made in the methods of detecting the sick animals. The officer and non-commissioned officer in charge of the animals were impressed with the importance of maintaining the same routine, in order that ensuing changes could be accurately assessed. Horses with ocular injuries of traumatic origin (keratitis, conjunctivitis) were brought to the hospital with about the same frequency during both years. It is certain that all cases of ocular disease were detected with at least as great fidelity during 1944 as in 1943. It is highly unlikely that any cases went undetected.

For the sake of convenience and accuracy, crystalline riboflavin was weighed on an analytical balance in the laboratory and placed in small, cork-stoppered vials which were covered with black paper. The correct amount to be added to each 100 lb. of grain was placed in each vial and, because the grain was received in 100-lb. sacks, this obviated reweighing the grain. The correct number of vials were emptied into a bucket about half full of grain and mixed thoroughly. This mixture was then thoroughly mixed with the rest of the grain in the feed rack. Feed for all horses in the stable was mixed in a bin, the riboflavin being added at this time.

At the start, riboflavin was added daily at the rate of 50 mg. per horse. On Jan. 1, 1944, this allowance was lowered to 40 mg. per horse and continued at this rate during 1944.

For controls, four sections of the depot were chosen. In these, a total of about 3,600 horses were being maintained under conditions similar to those of the experimental group in anticipation of issue to troops. All were mature horses. Procedures for the detection of sick and injured animals were essentially the same in all areas.

The animal strength of each group was recorded daily. The daily strength added together and divided by the number of days in the period (month or year) gave the mean daily strength. Admission rates, or new-case rates, were determined by the following formula[9]:

$$\text{Admission rate per 1,000, per annum} = \frac{\text{No. of cases} \times 1000 \times \text{theoretical duration (12 months).}}{\text{Mean strength} \times \text{actual duration of experience.}}$$

The effect of feeding riboflavin upon the incidence of periodic ophthalmia is graphically portrayed in table 8. Following the daily administration of riboflavin in rather

large doses (40 mg. per day), the appearance of new cases of the disease abruptly stopped.

A question arose in connection with the high incidence of periodic ophthalmia in this experimental group during 1943 and before the administration of riboflavin. Was this high rate due to conditions at Front Royal or to recurrences after arrival at Front Royal? Careful study of the records of the horses in which the disease developed during 1943 revealed that none had acute symptoms at Front Royal until the admission recorded in these experiments. It was found that these horses had been in this same group at Front Royal an average of eighteen months and twelve days before becoming affected with periodic ophthalmia. The most recent arrival admitted to the hospital had been at the depot five months and nineteen days. The other extreme was represented by a horse which had been there for sixty months and twenty-three days before the initial attack of periodic ophthalmia. It is highly probable that these 12 cases appearing in 1943 were the result of conditions prevailing at the Front Royal Depot.

In June, 1944, riboflavin supplementation was extended to include the nursery stock at the Front Royal Depot. By January, 1945, all animals at this depot (approximately 350) were receiving the enriched ration. A slightly different method of administration was started at this time which proved more satisfactory. Riboflavin was weighed in 40-Gm. lots and placed in black screw-top bottles. The contents of each bottle were mixed at the granary with 1,000 lb. of wheat bran. This was accomplished by mixing the bran in a pile on the granary floor. This was then mixed in the

TABLE 8—Effect of Riboflavin upon New Admissions for Periodic Ophthalmia

Experimental Group

	1943			1944		
Month:	Mean daily strength	No. of new cases	Annual rate /1000	Mean daily strength	No. of new cases	Annual rate /1000
Jan.	99.26	0	0	130.81	0	0
Feb.	90.71	2	264.57	127.69	0	0
March	90.90	2	264.02	126.29	0	0
April	102.70	2	233.69	118.23	0	0
May	109.52	0	0	115.94	0	0
June	110.07	1	109.02	115.43	0	0
July	110.68	0	0	115.90	0	0
Aug.	111.48	2	215.28	121.10	0	0
Sept.	114.17	1	105.09	120.37	0	0
Oct.	116.55	0	0	130.26	0	0
Nov.	125.67	2*	190.97	139.87	0	0
Dec.	132.52	0	0	148.71	0	0
Annual	109.52	12	109.56	125.88	0	0

Control Group

	1943			1944		
Month:	Mean daily strength	No. of new cases	Annual rate /1000	Mean daily strength	No. of new cases	Annual rate /1000
Jan.	3654.55	13	42.68	1389.38	10	86.39
Feb.	2934.61	3	12.26	767.07	3	46.91
March	2488.39	15	72.33	725.16	4	66.19
April	2437.30	13	64.00	667.27	1	17.97
May	2317.03	15	77.68	675.26	2	35.55
June	2339.63	12	61.54	779.80	0	0
July	2322.20	9	46.50	772.32	2	31.07
Aug.	2323.39	13	67.14	747.58	1	16.05
Sept.	2411.57	6	29.85	698.07	2	34.38
Oct.	2433.52	6	29.58	324.36	0	0
Nov.	2481.44	16	77.37	169.47	0	0
Dec.	2485.97	7	33.78	0†	0	0
Annual	2552.47	128	50.14	701.43	25	38.88‡

*Riboflavin feeding was started in the experimental group on Nov. 12, 1943.
†All animals were moved from these stables in November, 1944.
‡Based upon eleven months' experience.

usual manner by shoveling the bran first into one pile, then another. The yellow color of the riboflavin made it possible to determine when mixing was complete. The vitamin adhered to the bran with no tendency to settle out. This enriched bran was then resacked before issue to the various stables.

Since each pound of bran contained 40 mg. of riboflavin it was simple to regulate the amount fed. Most horses received 1 lb. of bran (in addition to other feed) which supplemented their rations with 40 mg. of riboflavin. Some broodmares and stallions received 2 lb. of enriched bran, which contained 80 mg. of riboflavin. Young animals on lush pastures were fed only 0.5 lb. of bran. When the pastures were particularly good, no grain of any kind was fed. As soon as the pastures became short and grain feeding was resumed, the feeding of enriched bran was again started. It appeared likely that horses on lush pastures (containing legumes) do not require additional riboflavin provided they have received large amounts at other times. This is one phase upon which further study will be required. The dosage was somewhat arbitrarily determined. Future experience may indicate that much less is required.

Not one new case of periodic ophthalmia developed among horses receiving the riboflavin supplement. The Veterinary Research Laboratory was transferred from Front Royal in September, 1945. At that time, no new cases had been seen in suplemented groups since July, 1944. At the present writing (March, 1946), reports from Front Royal indicate that riboflavin is being fed and no new cases have appeared. The animal strength at that station has remained above 300. The previous high incidence at Front Royal makes this absence of new cases particularly striking.

The cost of feeding riboflavin to horses is of importance. This vitamin is being manufactured on a large scale for use in enriching flour. In our work, riboflavin, U.S.P.*, was used entirely. At present, this is available in wholesale lots of 500 Gm. or more at 20 cents per gram. This would make the cost of feeding 40 mg. to one horse about 0.8 of a cent per day. A cheaper grade of riboflavin (85%) is now available, which is very satisfactory for animal feeding and costs only 13 cents per gram. Fed at the rate of 50 mg. per horse, the daily cost, using this grade, is about 0.6 of a cent, therefore, not prohibitive.

On the basis of this experience, the feeding of riboflavin as a preventive measure has been recommended for regions where periodic ophthalmia is prevalent[10].

*Our supplies were secured from two manufacturers: Merck & Company, Inc. Rahway, N. J., and Hoffman La-Roche, Nutley, N. J.

DISCUSSION

From the data presented in this report, it is apparent that riboflavin deficiency is not the only factor in the causation of periodic ophthalmia. However, the prompt and complete control of the disease following feeding of riboflavin and the nature of the lesions[1] indicates that this vitamin is involved in some way. Failure of riboflavin to arrest the course of the established disease is noteworthy. Vitamin A and niacin also proved ineffective in this respect.

The reasons for the effectiveness of large doses of riboflavin in the prevention of periodic ophthalmia are not understood although several explanations suggest themselves. Perhaps high riboflavin diets exert a protective influence upon ocular tissues; perhaps riboflavin, when present in ample amounts, acts as a "sparing agent" upon other nutritive elements essential to physiologic activity in the eyes. Such a phenomenon has been demonstrated in the case of niacin and tryptophane[11]. The absence of sufficient amounts of this essential amino acid was found to accentuate the effect of low niacin diets. Large amounts of niacin overcame this deficiency of tryptophane. This same possibility in the case of riboflavin and tryptophane is being studied further. Corn is especially deficient in tryptophane[12].

The rôle of essential amino acids in periodic ophthalmia deserves further attention.

The ocular lesions of tryptophane and lysine deficiencies have many similarities both to those of experimental riboflavin deficiency and periodic ophthalmia[13 to 16]. The interrelation of vitamins and other nutritive essentials is as yet imperfectly understood. These factors are being studied in this laboratory with special attention to their influence upon ocular disease.

The experience of the British Army in the South African War deserves study in connection with periodic ophthalmia. The official history of that war, written by Sir Fredrick Smith[17], indicates that this disease was a serious problem. Periodic ophthalmia was unknown in South Africa prior to the importation by the British of horses from other countries, particularly the United States. The disease did not become a serious problem until the end of the war in 1902, but then 25 per cent of the horses at some remount depots became involved, at others as high as 48 per cent. The Army of Occupation was estimated to have over 14 per cent of its animals affected with periodic ophthalmia. The disease was believed to be infectious but the infectious agent could not be demonstrated nor could transmission be proved. The disease declined after the war and eventually disappeared from South Africa. It did not become established in native animals.

The nutritional theory of causation sheds new light upon this experience. In this historical account, native horse-feeding practices were described as follows: "In Africa, a horse is allowed, according to the locality, from 10 to 15 acres for grazing all the year round, no other food being given". Feeding of the British military animals was sharply divergent from this practice. Throughout Sir Fredrick Smith's book are repeated references to the difficulties encountered in securing proper forage for military animals. Due to continuous shortage of military transport, horses were usually allowed a concentrate ration but were expected to make up the bulk by grazing. The concentrate ration consisted of "mealies" (Indian corn) and "mealie meal", for the most part, with oats and corn (wheat) occasionally supplied in small amounts. Wheat straw and chaff were sometimes available, but hay was seldom supplied. Grazing was usually very unsatisfactory, due to insufficient time, enemy activity, or lack of forage caused by the enemy's "scorched earth" policy. Not until after the war was it possible to bring the condition of the horses and mules up to the desired standard. In the remount depots, this was accomplished by more liberal feeding, especially of concentrates such as corn.

There are many pitfalls in drawing conclusions in retrospective study of limited data. However, speculation should be permissible provided it is clearly labeled as such.

To speculate: It is clear that the military ration described above was very deficient in riboflavin, as well as other factors. The high proportion of corn meant that tryptophane was also particularly lacking. This is in striking contrast to the feeding practices in South Africa prior to the war and subsequent to the removal of the British forces. Periodic ophthalmia did not spread to the native stock as would be expected were it an infectious disease. The inference is obvious: nutritional factors were responsible.

SUMMARY

Treatment of equine periodic ophthalmia (recurrent iridocyclitis) by feeding riboflavin, niacin, brewer's yeast, vitamin A, and dried skimmilk was found to be of no value in alleviating the acute attack or preventing recurrences. Limited trial of intravenously injected ascorbic acid indicated that there is possible value in this treatment.

Optimum conditions for the production of periodic ophthalmia were approximated but not attained. Low riboflavin diets did result in an increased incidence of periodic ophthalmia but not as high as was expected. The incidence of vascularizing keratitis was greatly increased. Further experimental study in this connection is needed.

The distribution of riboflavin and niacin in the equine body was studied by numerous analyses. Riboflavin is slightly decreased in the tissues of horses affected with periodic ophthalmia. The concentration is also decreased in horses fed a low riboflavin ration. Presumptive evidence is presented which indicates that riboflavin is synthesized by bacterial action in the equine intestinal tract. This action is most effective in horses fed a low riboflavin ration.

Analysis of equine feedstuffs revealed wide divergence in riboflavin concentration. It was clearly demonstrated that some rations are definitely low in riboflavin. This is affected by the quality of the feed and feeding practices followed.

Riboflavin was found to be of definite value in the prevention of new cases of periodic ophthalmia. Addition of 40 mg. of riboflavin daily to the feed of each horse was followed by immediate control of the disease. Although the Front Royal Remount Depot, at one time, had an extremely high incidence, no new cases have developed in the past two years among horses receiving riboflavin supplementation. This measure is recommended as a means of control.

ACKNOWLEDGMENTS

This investigational work was projected under the technical supervision of Brigadier General Raymond A. Kelser, Chief of the Veterinary Division, Office of the Surgeon General, U. S. Army, Washington, D.C. The continued advice and support of General Kelser formed an essential part of this work. The complete coöperation of Colonel William R. Wolfe, V. C., the depot veterinarian, was extremely important and is gratefully acknowledged.

The interest shown by many outstanding horsemen in the work of this laboratory has been a vital source of inspiration. Many of these people contributed horses to the laboratory which were highly valuable aids in our work. It is desired to thank the following persons in particular: Mr. Humphrey Finney, Towson, Md.; Mr. A. MacKay-Smith, White Post, Va.; Mr. Dan Wight, Frederick, Md.; Father Dennis, Rockcastle, Va.; Mr. Robert Sterling Clarke, New York, N. Y.; Dr. W. W. Dimock, Lexington, Ky.; Miss Julia Shearer, Locust Dale, Va.; Mr. Walter Chrysler, Jr., Warrenton, Va.; Mrs. George Greenhalgh, Berryville, Va.; Brigadier General Pleas B. Rogers, U. S. Army; Mr. Hackett, Darlington, Md.; Mr. Holland Potter, Upperville, Va.; Mr. W. A. Rhinehart, Charlottesville, Va.; Mr. Graham Dougherty, Berryville, Va., and Mrs. Mary E. Koegh, Orange, Va.

References

[1]Jones, T. C., Maurer, F. D., and Roby, T. O.: The Rôle of Nutrition in Equine Periodic Ophthalmia. Am. J. Vet. Res., *6*, (1945): 67-80.

[2]Roby, T. O., and Jones, T. C.: The Blood in Equine Periodic Ophthalmia. To be published.

[3]Morrison, F. B.: Feeds and Feeding. 20th ed. The Morrison Publishing Co., Ithaca, New York, 1943.

[4]Rosenberg, H. R.: Chemistry and Physiology of the Vitamins. Interscience Publishers, Inc., New York, 1942.

[5]Conner, R. T., and Straub, G. J.: Combined Determination of Riboflavin and Thiamin in Food Products. Ind. and Eng. Chem., (Anal. Ed.), *13*, (1941): 385.

[6]Snell, E. E., and Strong, F. M.: A Microbiological Assay for Riboflavin. Ind. and Eng. Chem., (Anal. Ed.) *11*, (1939): 346.

[7]Landy, M., and Dicken, D. M.: Microbiological Assay Method for Six B Vitamins Using *Lactobacillus Casei* and Medium of Essentially Known Composition. J. Lab. and Clin. Med., *27*, (1942): 1086-1092.

[8]Jones, T C.: Equine Periodic Ophthalmia. Am. J. Vet. Res. *3*, (1942): 45-71.

[9]Simmons, James S.: Laboratory Methods of the United States Army. 4th Ed. Lea and Febiger, Philadelphia, 1935.

[10]Kelser, R. A.: Preventing and Controlling Disease among Horses and Mules. An address before the 26th Annual Meeting of the Horse and Mule Association of America, Inc., Dec. 5 1945.

[11]Krehl, W. A., Teply, L. J., Sarma, P. S., and Elvehjem, C. A.: Growth-retarding Effect of Corn in Nicotinic Acid-low Rations and Its Counteraction by Tryptophane. Science, *101*, (1945): 489-490.

[12]Greene, R. D., and Black, A.: The Microbiological Assay of Tryptophane in Proteins and Foods. J. Biol. Chem., *155*, (1944): 1-8.

[13]Albanese, A. A.: Corneal Vascularization in Rats on a Tryptophane Deficient Diet. Science, *101*, (1945): 619.

[14]Totter, J. R., and Day, P. L.: Cataract and Other Ocular Changes Resulting from Tryptophane Deficiency. J. Nutr., *24*, (1942): 159-166.

[15]Albanese, A. A., and Busche, W.: On Cataract and Certain Other Manifestations of Tryptophane Deficiency in Rats. Science, *95*, (1942): 584-586.

[16]Sydenstricker, V. P., et al.: Amino Acid and Protein Deficiencies as Causes of Corneal Vascularization: A Preliminary Report. Science, *103*, (1946): 194-196.

[17]Smith, F.: Veterinary History of the War in South Africa. H. and W. Brown, London, 1919.

Experimental Treatment of Trichomonas Foetus Infection in Bulls

DAVID E. BARTLETT, D.V.M.
Beltsville, Maryland

IT HAS BEEN established that *Trichomonas foetus* infection in females is ordinarily self-curing but that bulls usually remain permanently infected. In view of these facts, it is obvious that, in any program designed to eliminate *T. foetus* infections from a herd, the first direct step is to stop further transmission of trichomonads from bulls through coitus. Elimination of infected bulls, if they have no exceptional value as sires, should be accomplished by slaughter. Infected bulls, however, are frequently very valuable because of the "blood lines" concentrated in them. *T. foetus* does not affect the potential ability of bulls to transmit desirable characteristics, nor does it affect, ordinarily, their fertility or potency. However, the trichomonads which they harbor, when transferred to susceptible heifers or cows at coitus, render them unproductive of calves for a variable period. An effective and practical method of either coping with or eliminating this infection in valuable bulls would restore their usefulness as sires.

One way in which attempts have been made to salvage some of the value of infected sires has been to confine their services exclusively to previously infected and, therefore, relatively resistant, females. Although this procedure affords a means of partial control of the disease and minimizes its effects, it has distinct disadvantages and limitations as follows: A dangerous reservoir of infection is maintained. Only selected females may be mated to the infected bulls. Results of exposing previously infected females are unpredictable because their previously acquired immunity is not absolute and eventual reinfection is probable.

Another method designed to restore trichomonad-infected bulls to usefulness is treatment with drugs designed to destroy the causative organism.

It is the purpose of this paper (1) to review briefly the literature pertaining to previously reported treatments for bovine venereal trichomoniasis in bulls, (2) to report the results of controlled experimental tests of several of the previously described treatments, and (3) to present preliminary results of original experimental treatments, utilizing a new approach, that have shown some promise in investigations so far conducted.

REVIEW OF THE LITERATURE

Cameron, Fincher, and Gilman (1933)[1] reported treating a bull by flushing the prepuce with 0.5 per cent Lugol's solution two or three times a week. Subsequently, this bull was bred to a cow and trichomonads were found in the vaginal mucus.

Abelein (1934)[2] reported on the treatment of infected bulls with disinfecting douches. Usually, these treatments were only "temporarily effective," or apparently effective, trichomonads usually becoming microscopically demonstrable when a thorough examination was made three weeks after treatment.

Brauer (1935)[3] recommended, but did not report the results of, douching the prepuce of infected bulls with 1:1,000 chinosol, entozon, therapogen, and valvanol solutions.

Söntgen (1935)[4] reported the treating of bulls by ballooning the preputial cavity with 1 : 1,000 entozon solution and then massaging vigorously for fifteen minutes, the treatment being applied daily for two to three weeks. His method of checking the results consisted

This work was carried out at the United States Department of Agriculture, Agricultural Research Center, Beltsville, Md.

The author is from the Zoölogical Division, Bureau of Animal Industry, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Md.

German references cited in this publication were translated by Dr. Gerard Dikmans of the Zoölogical Division. His contribution is gratefully acknowledged.

of test breeding eight to fourteen days after treatment. He reported that the greater number of bulls treated were apparently cured.

Küst (1935)[5] reported that recently infected bulls may be cured by intensive treatment with solutions or ointments, but that bulls with infections of long standing were incurable. This author (1936)[6] reported that recently infected bulls can be cured in most cases by repeated douching of the preputial cavity with 1 : 1,000 entozon or chinosol solution, or by the introduction of rods medicated with entozon, bissulin, or valvanol. In chronically infected bulls or bulls with infections of long standing, he reported that such treatment was usually ineffective. Küst believed treatment could be successful only when it was repeated several times on bulls that had been cast, under epidural anesthesia, but even then it could not be expected to be successful at all times. Many times, animals appeared to be cured and immediately following the treatment they did not transmit the infection; later, however, cows served by them became infected due to the fact, Küst believed, that the parasite was harbored inside the genital organs, probably in the urethra.

Möller (1936)[7] reported that treatment was more successful in recently infected bulls than in bulls with long standing infections. Repeated, thorough preputial douching with chinosol or entozon solutions (1 : 1,500) and the introduction of disinfecting rods into the prepuce while the animal was in the standing position were not always successful in chronically infected bulls. In recently infected bulls, these measures were found more successful but, even in these cases, a complete cure could not always be guaranteed.

Benesch and Wurst (1936)[8] reported that they found trichomonads in the prepuce of a bull after he had been treated four times in four weeks with entozon solution.

Gould (1937)[9] reported douching bulls with 5 per cent lactic acid but presented no specific results.

Schmidt (1937)[10] reported treating several bulls. While the animals were under epidural anesthesia, he applied 1.5 per cent oxyquinoline sulfate solution to the genital membranes. No post-treatment examinations or tests were reported for these bulls but, according to this author, the "inflammatory lesions that existed preceding treatment decreased following treatment."

Schmidt, Turk, and Shephardson (1937)[11] reported the treating of several bulls by thoroughly washing the penis with warm 5 per cent solution of oxyquinoline sulfate every eight to ten days for nine months. Breeding operations were resumed three months after the beginning of the treatment. No post-treatment examinations or tests were reported for these bulls, but according to the authors, all the cows bred to these previously infected bulls promptly conceived; in none could the presence of trichomoniasis be established. The following year, these authors[12] reported that 3 of the bulls were still being used in the herd. Later (1939, 1940, 1941) they reported no further positive cases.[13, 14, 15]

Abelein (1938 and 1941),[16, 17] and Swangard (1938, 1939, and 1941)[18, 19, 20] separately reported treatment of trichomoniasis of bulls, while cast and under epidural anesthesia, by application of trypaflavine ointment to the penile and preputial membranes, and by douching the anterior urethra with trypaflavine solution. Abelein's (1941) publication reports that 122 bulls (19 suspected of being infected and 103 definitely infected) were treated by rubbing various concentrations of trypaflavine in an ointment base into the penis and preputial cavity and by injecting trypaflavine solution into the urethra with the following results: (1) Twenty-four bulls were treated with "bovoflavinsalve" in which the trypaflavine concentration was greater than 0.5 per cent and with trypaflavine solution stronger than 0.1 per cent. Twenty of these bulls were cured and 4 had to be disposed of because of damage resulting from the treatment. (2) Fifty-seven bulls were treated with "bovoflavinsalve" in which the trypaflavine concentration was 0.5 per cent and with 0.1 per cent trypaflavine solution; 55 of these bulls proved to be cured on examination two to four weeks later and 2 were not cured. (3) Thirteen bulls were treated with "bovoflavinsalve" in which the trypaflavine concentration was 0.4 per cent and with 0.1 per cent trypaflavine solution; 11 of these bulls were cured and 2 were not cured. (4) Nine bulls were treated with "bovoflavinsalve'" in which the trypaflavine concentration was 0.3 per cent and with 0.1 per cent trypaflavine solution; 8 of these bulls were cured and 1 was not cured.

Abelein, who followed the results of services of 29 of these bulls, stated that they did not infect any cows and proved to be good sires, the cows bred to them calving normally. Three months or more after treatment, 4 out of 14 community bulls and 5 out of 15 privately owned bulls "relapsed". Abelein believed that probably they became reinfected because there was no control over the females they served. He outlined an adequate procedure to test the post-treatment status of bulls but was unable, in many instances, to carry out the procedure because the bulls were not under constant ob-

servation. His testing plan consisted of: culturing the preputial secretions, microscopic examinations of the preputial secretions, observation of females after one or more test services, and watching over the results after the bull was returned to service and over the females bred to him.

Kerr (1943)[21] reported the failure of brilliant green (0.1% for penis and prepuce and 0.01% for urethra) to effect a cure in 1 bull.

Wright (1945)[22] reported an unsuccessful treatment of a bull from the application of 0.1 per cent metaphen ointment to the penis.

The preceding review of published reports on the treatment of trichomoniasis in bulls makes evident the necessity of further investigations of the problem. Some reports are sketchy; many are conflicting. About half of the workers reported failures rather than successes as the results of their efforts to eliminate the infection from bulls. No worker who treated more than a single bull reported consistent success. Several of the bulls reported as cured of the infection were judged by criteria that are obviously inadequate. A reasonable doubt as to the validity of some claims of cures is, therefore, justified. For example, some workers have presented, as evidence of the effectiveness of treatment, the observation that after treatment of the bull and sexual rest of the infected cows, coitus resulted in normal pregnancies. This has little bearing upon the infectious status of the bull, as the females that have recovered from their infection may have acquired resistance to reinfection. Neither is it significant that preputial samples collected for a week or two following treatment contained no *T. foetus*. It is usual for the organisms not to appear for several weeks or longer subsequent to unsuccessful treatment.

EXPERIMENTAL PROCEDURE

Nine bulls (A99, 193, 207, 208, 214, 250, 266, 274, and 275), infected with *T. foetus*, were used in the present investigations. Two of these (208 and 214), after being cured of one infection by treatment, were reinfected experimentally[23] and again treated. Eight other animals (6 males and 2 females) were used exclusively in pharmacologic studies of iodine compounds.

All work involving animals other than bulls 266, 274, and 275 was carried out at the Agricultural Research Center, Beltsville, Md. These bulls received two, two, and three treatments, respectively, on Maryland farms prior to being brought to Beltsville.

Two distinct types of treatment procedures are reported in this paper. These are:

1) *Direct Application of Trichomonadicidal Agents to Genital Membranes as Douches or Ointments.*—The douches were administered with a pressure pump. The prepuce was tightly ballooned and massaged several times during the ten to fifteen minutes spent in the operation. Before applying ointments, anesthesia of the genital membranes and partial paralysis of the retractor penis muscle were produced by epidural injections of procaine solution[24]. With the bull in the standing position, the penis was withdrawn, the ointment rubbed into the membranes of the prepuce and glans for ten to fifteen minutes, and the urethra flushed with antiseptic solution.

2) *Oral or Intravenous Administration of Iodine Compounds, so that Iodine* Indirectly Reaches the Genital Membranes.*—Potassium iodide was administered orally in capsules with an ordinary balling gun. Intravenous injections of aqueous sodium iodide solutions were made with a standard gravity flow apparatus. In order to obviate the chance of accidentally infiltrating the perivascular tissue as a result of the needle slipping out of the jugular vein, a 6-inch, flexible 18-gauge, blunt needle (calcium chloride needle) was passed through the needle used to puncture the vein. Prior infiltration of the skin and subcutis with a 2 per cent procaine solution in the region of the puncture prevented much of the restlessness which ordinarily occurs during intravenous injections.

Chemical tests demonstrated that iodine was present in appreciable concentration on the preputial and penile membranes and that it was also present in the semen, saliva, tears, urine, milk, and blood following administration of large doses of iodides.

The exact chemical form in which the iodine appeared is not yet known. The reagents used in these tests converted the iodine to its molecular form. The tests used were qualitative, but it was possible to make some approximate quantitative estimates of the levels of iodine concentration. The tests used were as follows:

1) To a quantity up to 5 cc. of the sample, 2 cc. of carbon tetrachloride, 5 cc. of 20 per cent sulfuric acid, and 2 cc. of 0.2 per cent sodium nitrite were added and the mixture shaken thoroughly. The presence of molecular iodine was indicated by the distinctive, pink color which resulted when it was dissolved by carbon tetrachloride.

2) To a quantity up to 5 cc. of the sample were added 5 cc. of 20 per cent sulfuric acid,

*The iodine compound is at present unidentified. Hereafter, in this paper, the word iodine is used to designate this unknown iodine compound.

TABLE I—Unsuccessful Experimental Treatments of Trichomonas Foetus Infection in Bulls by Application of Trichomonadicidal Agents to the Glans Penis, Prepuce, and Lower Urethra

Animal number	Trial number	Date treated	Method employed	Trichomonadicidal agent	Time of appearance of trichomonads after treatment
	1	12-4-39 12-11-39	Preputial douche	†Aqueous solution ?% sodium perborate	1st week
	2	12-?-39	Preputial douche	†Aqueous solution 3% agent A	1st week
	3	1-22-40	Preputial douche	†Aqueous solution 5% agent A	3rd week
*A99	4	5-6-40 5-9-40 5-13-40 5-16-40 5-20-40 5-23-40 5-27-40	Preputial douche	†1% Lugol's solution	Positive during treatment
	5	8-7-40 8-13-40 8-17-40 8-20-40 8-2?-40	Preputial douche	450 cc. 1% Lugol's solution	1st week
	6	9-17-40 9-18-40 9-19-40 9-20-40 9-21-40	Preputial douche	450 cc. 5% Lugol's solution	1st week
193	1	1-13-43	Preputial douche	3 liters 0.1% aqueous solution agent B	2nd week
	1	11-12-42	Epidural anesthesia-jelly applied to glans penis and prepuce; urethral douche	50 cc. 15% agent B jelly; 30 cc. 0.1% aqueous solution agent B	1st week
	2	12-10-42	Preputial douche	3 liters 1% aqueous solution agent B	6th week
208	3	2-18-43	Epidural anesthesia-ointment applied to glans penis and prepuce; urethral douche	50 cc. 0.1% agent C in ointment base I; 30 cc. 0.02% aqueous solution agent C	2nd week
	4	4-5-43 4-6-43 4-7-43 4-8-43 4-9-43 4-10-43	Preputial douche	3 liters 0.05% aqueous solution agent D	1st week
	5	6-9-43	Ointment forced under pressure into prepuce	500 cc. 0.1% agent C in ointment base I	1st week
274	1	5-7-45 5-16-45	Epidural anesthesia-ointment applied to glans penis and prepuce; urethral douche	100 cc. 0.2% agent E in ointment base II; 20 cc. 0.1% agent E	3rd week

*A99—Experiments on this bull were planned and carried out by George G. Garlick who resigned from the Zoölogical Division, May, 1941.

†—Quantity not recorded.

Commercial preparations used:

Trichomonadicidal agent A—Sodium hypochlorite = Zonite
Trichomonadicidal agent B—Sodium dioctyl sulfo-succinate = "Aerosol OT 100%"
Trichomonadicidal agent C—Sodium ethyl mercuri thiosalicylate = "Merthiolate"
Trichomonadicidal agent D—Alkyl dimethyl benzyl ammonium chlorides = "Zephiran Chloride"
Trichomonadicidal agent E—3.6. Diamino—10 methyl acridinium chloride = "Trypaflavine". The ointment was furnished by Dr. W. M. Swangard, Eugene, Ore.

Ointment base I —High molecular weight polyethylene glycols = "Carbowax 1500"

Ointment base II—Unknown. Furnished by Swangard.

2 cc. of 0.2 per cent sodium nitrite, and then, quickly, a few drops of corn starch solution. The presence of molecular iodine was indicated by the distinctive, deep-blue color of starch iodide, a product of the reaction.

Summarized, the pertinent facts determined by testing material obtained from the prepuces of bulls receiving iodides were:

1) Intravenous administration of each dose of sodium iodide, at the rate of 30 to 50 Gm. per 1,000 lb. of body weight, was followed by the appearance of iodine in the prepuce in considerable concentration for forty-eight to seventy-two hours.

2) Oral administration daily of doses of potassium iodide, at the rate of 10 Gm. per 1,000 lb. of body weight, was followed by the appearance of iodine in the prepuce in limited concentration on the seventh or eighth day. Throughout the remainder of the period of administration, and for four to five days subsequently, iodine was present in variable limited concentrations.

RESULTS

Table 1 summarizes 13 unsuccessful experimental treatments of 4 bulls with douches or ointments. Seven active trichomonadicidal agents prepared either in aqueous solution or incorporated in one of two ointment bases were used in these tests. The materials used were: (a) disinfectants — sodium perborate, Lugol's solution, and five proprietary compounds whose chemical formulas are given in the table, (b) ointment bases — two proprietary compounds — the chemical formula of one is given in the table, that of the other is unknown.

Table 2 shows six successful chemotherapeutic treatments and 15 unsuccessful treatments involving 8 infected bulls. Two of these were experimentally reinfected subsequent to recovery and re-treated. Four different chemotherapeutic procedures were tried. They are presented in the order studied.

1) *Potassium Iodide Orally.*—Daily oral administration of potassium iodide at the rate of approximately 10 Gm. per 1,000 lb. of body weight for twenty-three to twenty-five days cured the first 2 bulls so treated. This procedure had the disadvantage of requiring frequent handling of the subject. Chemical tests of the preputial material revealed that more than a week elapsed before iodine appeared.

2) *Sodium Iodide Intravenously.*—Given intravenously in a few large doses, usually a total of three to five doses at two or three-day intervals at the rate of 30 to 50 Gm. per 1,000 lb. of body weight, sodium iodide seemed more practical than potassium iodide given orally in many small doses. Chemical tests revealed that iodine appeared in the preputial material a half to two hours after administration. However, of the first series of five infections so treated, only the first and last were cured. Subsequent treatments of seven infections resulted in only one additional cure.

3) *Sodium Iodide Intravenously Supported by Potassium Iodide Orally.*—Combination treatment consisting of two intravenous doses of sodium iodide, at the rate of 30 Gm. per 1,000 lb. of body weight, separated by a three-day interval, and followed by daily oral administration of potassium iodide at the rate of 8 to 10 Gm. per 1,000 of body weight for thirty days, proved unsuccessful in two instances.

4) *Sodium Iodide Intravenously plus Douches with an Oxidizing Agent.*—It was recognized that the form of iodine present in the prepuce following administration of sodium iodide was not an active trichomonadicide. *In vitro* tests showed that molecular iodine, an active trichomonadicide, was released by the addition of a small quantity of weak chloramine-T solution to the preputial secretions of treated bulls. Of 5 bulls treated experimentally with a combination of sodium iodide intravenously plus chloramine-T douches, 1 was cured.

Throughout all of these experiments, genital specimens were collected regularly from both females and males and examined microscopically for trichomonads in accordance with techniques previously described[28].

In these experiments, final judgment of the effectiveness of each treatment in curing an infected bull was based essentially upon: (1) consistently negative weekly

post-treatment examinations of preputial samples for not less than nine months up to one year; (2) normal reproduction by susceptible females as a result of coitus occurring at least nine weeks after treatment, each female having consistently negative vaginal samples (examined six days each week) beginning prior to coitus and continuing *post partum* at least three months. Five bulls reported cured have satisfied all of the above requirements. One bull (275), as shown in the protocols, has satisfied the first requirement but not all of the second requirement at the time of preparing this paper.

PROTOCOLS

In the following protocols, the histories, regimens, and results of treatments of the 8 bulls that received experimental iodine therapy are presented. The same data are summarized in table 2.

• • •

Bull 214, of mixed breed, born Dec. 3, 1940, was infected by means of the experimental technique previously described.[23] He was first inoculated on Aug. 11, 1942. Trichomonads were found at five of the six subsequent weekly examinations, but were not found in specimens collected from Oct. 1, 1942, to Dec. 8, 1942. On the later date, he was again inoculated. Organisms were present in nine of 15 samples taken between that date and May 15, 1943. During the period of infection, he served no susceptible females. No attempts to free this bull of infection were made before these experiments were undertaken.

First Treatment with Potassium Iodide (at Beltsville).—Administration orally of 8 Gm. per day of potassium iodide in capsules was begun on May 15, 1943, on which day the bull weighed approximately 800 lb., and continued through June 8, 1943. For the first eight days, half of the dose was given in the morning and the other half in the afternoon. For the remainder of the period, a single dose was given. A total of 200 Gm. of the drug was administered during the 24-day period.

Observations.—(1) Preputial samples were examined at least once each week from May 15, 1943, until July 10, 1944. Trichomonads were last found on May 22, 1943, the eighth day of treatment. Subsequent examinations were negative.

2) On Nov. 12, 1943, the bull served female 231, a virgin heifer. She returned to estrum on Dec. 2, 1943, and was again served. This second coitus initiated a normal pregnancy which terminated on Sept. 11, 1944, by the birth of a normal male calf. On Jan. 8, 1944, he served female 239, a virgin heifer. This coitus initiated a normal pregnancy which was terminated on Oct. 24, 1944, by the birth of a normal female calf.

3) Vaginal specimens from each of the 2 females were examined each day from the respective days of first coitus through the postpartum periods. In no instances were trichomonads present.

Result.—Treatment successful.

Reinfection.—On July 10, 1944, and July 13, 1944, the bull (214) was inoculated with *T. foetus* obtained from an infected female in accordance with the technique previously described.[23]

First Treatment with Sodium Iodide (at Beltsville).—Administration intravenously of sodium iodide was begun on Jan. 22, 1945, on which day the bull weighed 1,140 lb. Forty-five Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Jan. 22, 24, and 26, 1945, and on Feb. 12, 14, and 16, 1945. Sixty Gm. were given on March 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24, 1945. A total of 135 Gm. of the drug was administered during the first four-day period, and after an interval of seventeen days, 135 Gm. were administered during a second four-day period. After another interval of eighteen days 600 Gm. were administered during an eighteen-day period. Thus, a total of 870 Gm. of the drug was administered over a 62-day period.

Observations.—Trichomonads were found on examination subsequent to the second post-treatment week.

Result.—Treatment unsuccessful.

Second Treatment with Sodium Iodide plus Chloramine-T Douches (at Beltsville).—Administration of sodium iodide intravenously was begun on June 19, 1945, on which day the bull (214) weighed approximately 1,140 lb. Fifty Gm. of sodium iodide in 500 cc. of sterile distilled water were given on June 19, 21, 23, 25, and 27, 1945. A total of 250 Gm. of the drug was administered during the eight-day period. Administration of 0.25 per cent chloramine-T douches (2 liters) daily was begun on June 19, 1945, and continued through June 30, 1945. The sodium iodide was administered in the morning and the douche in the afternoon.

Observations.—Trichomonads were found on examinations subsequent to the second post-treatment week.

Result.—Treatment unsuccessful.

Third Treatment with Sodium Iodide plus Chloramine-T Douches (at Beltsville).— Ad-

ministration of sodium iodide intravenously was begun on Oct. 18, 1945, on which day the bull (214) weighed 1,140 lb. Fifty-five Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Oct. 18, 20, 22, 24, and 26, 1945. A total of 275 Gm. of the drug was administered during the eight-day period. Administration of 0.25 per cent chloramine-T douches (2 liters) daily was begun on October 18 and continued through Oct. 29, 1945. The sodium iodide was administered in the morning and the douche in the afternoon.

Observations.—Trichomonads were found subsequent to the fifth post-treatment week.

Result.—Treatment unsuccessful.

• • •

Bull 208, a purebred Guernsey, born Oct. 29, 1943, became infected while in a South Carolina dairy herd. His breeding record in that herd indicated that he became infected several years before being brought to Beltsville in August, 1940. Organisms were present in 107 of 136 preputial samples examined between Sept. 12, 1940, and June 15, 1943; during this period, he transmitted infections through coitus to 7 females. During this same period, five unsuccessful attempts were made to free this bull of infection with medicated douches and ointments. Details regarding these experiments are shown in table 1.

First Treatment with Potassium Iodide (at Beltsville).—Administration orally of 12 Gm. per day of potassium iodide in capsules was begun on June 15, 1943, on which day the bull (208) weighed 1,360 lb., and continued through July 7, 1943. A total of 276 Gm. of the drug was administered during the 22-day period.

Observations.—(1) Preputial samples were examined at least once each week from June 15, 1943, until Sept. 23, 1944. Trichomonads were last found on July 2, 1943, the eighteenth day of treatment. Subsequent examinations were negative.

2) On Nov. 22, 1943, the bull served female 181, a cow that was probably resistant to trichomonad infection. This coitus initiated a normal pregnancy which was terminated on Aug. 21, 1944, by the birth of a normal female calf. On Dec. 9, 1943, he served female 259, a virgin heifer. This coitus initiated a normal pregnancy which was terminated on Sept. 21, 1944, by the birth of a normal male calf. On Jan. 1, 1944, he served female 257, a virgin heifer. She returned to estrum on Jan. 20, 1944, and was again served. This second service initiated a normal pregnancy which was terminated on Oct. 29, 1944, by the birth of a normal male calf.

3) Vaginal specimens from each of these 3 females were examined on each week day from the respective days of first coitus through the postpartum periods. In no instance were trichomonads present.

Result.—Treatment successful.

Reinfection.—On Sept. 28, 1944, the bull was inoculated with *T. foetus* obtained from an infected bull in accordance with the technique previously described.[23] Reinfection developed.

First Treatment with Sodium Iodide (at Beltsville).—Administration of sodium iodide intravenously was begun on May 8, 1945, on which day the bull (208) weighed 1,380 lb. Sixty-five Gm. of sodium iodide in 500 cc. of sterile distilled water were given on May 8, 10, 12, 14, 16, 18, and 21, 1945. A total of 455 Gm. of the drug was administered during the thirteen-day period.

Observations.—Trichomonads were found on examinations subsequent to the second post-treatment week.

Result.—Treatment unsuccessful.

Second Treatment with Sodium Iodide plus Chloramine-T douches (at Beltsville).—Administration of sodium iodide intravenously was begun on Oct. 18, 1945, on which day the bull (208) weighed 1,360 lb. Sixty-five Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Oct. 18, 20, 22, 24, and 26, 1945. A total of 325 Gm. of the drug was administered during the eight-day period. Administration of 0.25 per cent chloramine-T douches (2 liters) daily was begun on Oct. 18, 1945, and continued through Oct. 29, 1945. The sodium iodide was administered in the morning, and the douche in the afternoon.

Observations.—Trichomonads were found on examinations subsequent to the third post-treatment week.

Result.—Treatment unsuccessful.

• • •

Bull 193, a grade Guernsey, born April 30, 1939, was infected experimentally as previously reported.[23] He was inoculated on April 13, 1942. Organisms were present in 52 of 66 preputial samples examined between the day of inoculation and July 16, 1943. During this period, one unsuccessful attempt was made to free this bull of infection with a medicated douche. Details regarding this treatment are presented in table 1. During this same period, the bull (193) transmitted infection through coitus to 7 females.

First Treatment with Sodium Iodide (at Beltsville).—Administration intravenously of sodium iodide was begun on July 16, 1943, on which day he weighed 1,585 lb. Fifty Gm. of sodium iodide in 1 liter of sterile distilled water were given intravenously on July 16,

TABLE 2—Experimental Treatments of Trichomonas Foetus Infection with Sodium Iodide and Potassium Iodide

Bull number	Trial number	Weight in lb. at beginning of treatment	Dose; drug: route; frequency; total doses	Result of treatment	Time of appearance of trichomonads after treatment
214	1	800	8 Gm. KI -O- daily; 25 doses	Success	....
214 Second infection	1	1,140	45 Gm. NaI -V- on alternate days; 3 doses. Interval of 17 days, 45 Gm. NaI -V- on alternate days; 3 doses. Interval of 18 days, 60 Gm. NaI -V- on alternate days; 10 doses.	Failure	2nd week
	2	1,160	50 Gm. NaI -V- on alternate days; 5 doses. 0.25 per cent chloramine-T douches daily; 12 douches.	Failure	2nd week
	3	1,140	55 Gm. NaI -V- on alternate days; 5 doses. 0.25 per cent chloramine-T douches daily; 12 douches.	Failure	5th week
208	1	1,360	12 Gm. KI -O- daily; 23 doses	Success	....
208 Second infection	1	1,380	65 Gm. NaI -V- on alternate days except for interval preceding last dose which was two days; 7 doses.	Failure	2nd week
	2	1,360	65 Gm. NaI -V- on alternate days; 5 doses. 0.25 per cent chloramine-T douches daily; 12 douches.	Failure	4th week
193	1	1,585	50 Gm. NaI -V- at 3-day intervals; 3 doses.	Success	....
250	1	1,790	50 Gm. (except for 4th dose which was 25 Gm.) NaI -V- at 3-day intervals; 5 doses.	Success	....
266	1	1,600	41 Gm. NaI -V- at 3-day intervals; 3 doses.	Failure	3rd week
	2	1,650	50 Gm. NaI -V- at 3-day intervals; 2 doses. 12 Gm. KI -O- daily; 30 doses.	Failure	4th week
	3	1,650	75 Gm. NaI -V- at 3-day intervals; 5 doses.	Success	....
274	1	1,800	48 Gm. NaI -V-; 1 dose. Interval of 3 days, 54 Gm. NaI -V- at 3-day intervals; 4 doses.	Failure	2nd week
	2	1,800	80 Gm. NaI -V- on alternate days; 3 doses. Interval of 9 days, 80 Gm. of NaI -V-; 1 dose.	Failure	8th week
	3	1,680	70 Gm. NaI -V- on alternate days; 5 doses. 0.25 per cent chloramine-T douches daily; 12 douches.	Failure	3rd week
275	1	1,000	30 Gm. NaI -V- at 3-day intervals; 4 doses.	Failure	7th week
	2	1,000	30 Gm. NaI -V- at 3-day intervals; 2 doses. 12 Gm. KI -O- daily; 30 doses.	Failure	3rd week
	3	1,000	45 gm. (except for 4th dose which was 25 Gm.) NaI -V- at 3-day intervals; 5 doses.	Failure	5th week
	4	1,140	50 Gm. NaI -V- on alternate days; 5 doses. 0.25 per cent chloramine-T douches daily; 10 douches.	Success	....
207	1	1,450	60 Gm. NaI -V- 1 dose. Interval of 2 days, 55 Gm. NaI -V- at 2-day interval; 2 doses. Interval of 17 days, 60 Gm. NaI -V- on alternate days; 3 doses.	Failure	2nd week
	2	1,450	65 Gm. NaI -V- on alternate days; 5 doses. 0.25 per cent chloramine-T douches daily; 12 douches.	Failure	2nd week

-V- = intravenously
-O- = orally

19, and 22, 1945. A total of 150 Gm. of the drug was administered during the six-day period.

Observations.—(1) Preputial samples were examined at least once each week from July 16, 1943, until July 21, 1944. Trichomonads were not found at any examination subsequent to the beginning of treatment.

2) On Sept. 3, 1943, the bull served female 198, a previously uninfected cow. This coitus initiated a normal pregnancy which was terminated on June 16, 1944, by the birth of a normal male calf. On Dec. 17 and 18, 1943, he served female 258, a virgin heifer. Approximately fifty days following coitus, a diagnosis of pregnancy was made from an examination of the internal genitalia *per rectum*. On March 19, 20, 21, and 22, 1944, considerable thick, yellow pus was found in the vagina. On March 22, 1944, on which day the heifer was in estrum, an examination *per rectum* revealed a nonpregnant uterus. April 12, 1944, she was again served by the same bull (193). A pregnancy did not develop from this coitus. Two months later, a single service by an infected bull produced a typical trichomonad infection in this heifer.

3) Vaginal specimens from female 198 were examined on each week day from the day of coitus through the postpartum period. In no instance were trichomonads present. Vaginal specimens from female 258, examined on each week day from the day of first coitus to the day of service by the infected bull, contained no *T. foetus*.

4) On July 18, 1944, the bull (193) was slaughtered. Examination of the glans penis, prepuce, urethra, seminal vesicles, vasa deferentia, epididymides, and testicles revealed no trichomonads.

Result.—Treatment successful.

• • •

Bull 250, a purebred Holstein-Friesian, born Aug. 30, 1940, was infected by means of the experimental technique previously described.[23] He was inoculated on Sept. 15 and 18, 1943; organisms were present in 12 of 21 samples examined between inoculation and Feb. 19, 1944. During the period of infection, no treatment attempts were made and no susceptible females were served.

First Treatment with Sodium Iodide (at Beltsville).—Administration intravenously of sodium iodide was begun on Feb. 22, 1944, on which day the bull (250) weighed 1,790 lb. Fifty Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Feb. 22, 25, and 28, 1944, 25 Gm. were given on March 2, and 50 Gm. on March 5, 1944. A total of 225 Gm. of the drug was administered during the 12-day period.

Observations.—(1) Preputial samples were examined at least once each week from Feb. 22, 1944, until Jan. 10, 1945. Trichomonads were not found at any examination subsequent to the beginning of treatment.

2) On May 11, 1944, the bull (250) served female 264, a virgin heifer. This coitus initiated a normal pregnancy which was terminated on Feb. 17, 1945, by the birth of a normal male calf. On July 2, 1944, he served female 263, a virgin heifer. On Aug. 8 and 31, and Sept. 19, 1944, this heifer was again served by the same bull. During this period of breeding, a purulent vaginitis existed. A pregnancy, initiated by the last service, terminated by abortion on Nov. 17, 1944. Bacteriologic examination of the embryo proved negative.* Four months later, a single service by an infected bull produced a typical trichomonad infection in this heifer. On Sept. 21, 1944, the bull (250) served female 198, a previously uninfected cow. This coitus initiated a normal pregnancy terminating July 8, 1945, by the birth of a normal male calf.

3) Vaginal specimens from each of the 2 females experiencing normal pregnancies were examined on each week day from the respective dates of first coitus through the postpartum periods. In no instance were trichomonads present. Vaginal specimens from the other female (263) were examined on each week day beginning on the day of first coitus, continuing through the period of breeding, pregnancy, and postabortion period. In no instance were trichomonads present.

4) On Jan. 10, 1944, the bull (250) was slaughtered. Examination of the glans penis, prepuce, urethra, seminal vesicles, vasa deferentia, epididymides, and testicles revealed no trichomonads.

Result.—Treatment successful.

• • •

Bull 266, a purebred Holstein-Friesian, born in August, 1940, became infected while in a Maryland dairy herd. His breeding history in that herd indicates that he had been infected several years before these experiments began. Between Sept. 11 and Nov. 2, 1943, trichomonads were present in four of five preputial samples examined. Trichomonads were found in specimens from several females served by this bull. No previous attempts at treatment had been made.

First Treatment with Sodium Iodide (at the farm).—Administration, intravenously, of sodium iodide was begun on Nov. 2, 1943, on which day the bull (266) weighed approxi-

*Bacteriologic examinations were made by Dr. C. A. Manthei, Pathological Division, BAI.

mately 1,600 lb. Forty-one Gm. of sodium iodide in 850 cc. of sterile distilled water were given on Nov. 2, 5, and 8, 1943. A total of 123 Gm. of the drug was administered during the six-day period.

Observations.—Trichomonads were found on examinations subsequent to the third post-treatment week.

Result.—Treatment unsuccessful.

Second Treatment with Sodium Iodide and Potassium Iodide (at the farm).—Administration of iodine compounds was begun March 15, 1944, on which day the bull weighed approximately 1,650 lb. Fifty Gm. of sodium iodide in 500 cc. of sterile distilled water were given intravenously on March 15 and 18, 1944. Twelve Gm. of potassium iodide, in capsules, were given orally on each day from March 19 to April 17, 1944. A total of 100 Gm. of sodium iodide and 360 Gm. of potassium iodide was administered over the 33-day period.

Observations.—Trichomonads were found on examinations subsequent to the fourth post-treatment week.

Result.—Treatment unsuccessful.

Third Treatment with Sodium Iodide (at Beltsville).—Administration intravenously of sodium iodide was begun July 15, 1944, on which day the bull (266) weighed 1,650 lb. Seventy-five Gm. of sodium iodide in 1 liter of sterile distilled water were given on July 15, 18, 21, 24, and 27, 1944. A total of 375 Gm. of the drug was administered during the twelve-day period.

Observations.—(1) Preputial samples were examined at least once each week from July 15, 1944, until April 2, 1945. Trichomonads were not found at any examination subsequent to the beginning of treatment.

2) On Oct. 21, 1944, the bull served female 235, a virgin heifer. This coitus initiated a normal pregnancy which terminated on July 23, 1945, by the birth of a normal female calf. On Dec. 13, 1944, he served female 238, a virgin heifer. This coitus initiated a normal pregnancy which terminated on Sept. 14, 1945, by the birth of a normal male calf.

3) Vaginal specimens from each of these 2 females were examined on each week day from the respective days of coitus through the postpartum periods. In no instance were trichomonads present.

4) On April 2, 1945, the bull (266) was slaughtered. Examination of the glans penis, prepuce, urethra, seminal vesicles, vasa deferentia, epididymides, and testicles revealed no trichomonads.

Result.—Treatment successful.

Bull 274, a purebred Aberdeen Angus, born March 22, 1937, became infected while in a Maryland beef herd. His breeding history in that herd indicated that he had been infected several months before these experiments were begun. Between Nov. 11, 1943, and Dec. 3, 1943, trichomonads were present in three of four preputial samples examined. Trichomonads were found in specimens from several females served by this bull. No previous attempts at treatment had been made.

First Treatment with Sodium Iodide (at the farm).—Administration intravenously of sodium iodide was begun on Dec. 3, 1943, on which day this bull weighed approximately 1,800 lb. Forty-eight Gm. of sodium iodide in 800 cc. of sterile distilled water were given on Dec. 3, 1943, and 54 Gm. were given in 900 cc. of water on Dec. 6, 9, and 12, 1943. A total of 210 Gm. of the drug was administered during the nine-day period.

Observations.—Trichomonads were found on examinations subsequent to the second post-treatment week.

Result.—Treatment unsuccessful.

Second Treatment with Sodium Iodide (at the farm).—Administration intravenously of sodium iodide was begun on Nov. 22, 1944, on which day the bull weighed approximately 1,800 lb. Eighty Gm. of sodium iodide in 1 liter of sterile distilled water were given on Nov. 22, 24, 26, and Dec. 5, 1944. A total of 240 Gm. of the drug were administered over the four-day period followed by a single dose of 80 Gm. after an interval of nine days.

Observations.—Trichomonads were found on examinations subsequent to the eighth post-treatment week.

Result.—Treatment unsuccessful.

Third Treatment with Sodium Iodide plus Chloramine-T Douches (at Beltsville).—Administration of sodium iodide intravenously was begun on Oct. 18, 1945, on which day the bull weighed 1,680 lb. Seventy Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Oct. 18, 20, 22, 24, and 26, 1945. A total of 350 Gm. of the drug was administered during the eight-day period. Administration of 0.25 per cent chloramine-T douches (2 liters) daily was begun on October 18 and continued through Oct. 29, 1945. The sodium iodide was administered in the morning, the douche in the afternoon.

Observations.—Trichomonads were found on examinations subsequent to the third post-treatment week.

Result.—Treatment unsuccessful.

Bull 275, a purebred Aberdeen Angus, born Jan. 25, 1942, became infected while in a Maryland beef herd. His breeding history in that herd indicated that he had been infected several months before these experiments were begun. Between Nov. 11, 1943, and Dec. 3, 1943, trichomonads were present in two of three preputial samples examined. Trichomonads were found in specimens from several females served by this bull. No previous attempts at treatment had been made.

First Treatment with Sodium Iodide (at the farm).—Administration intravenously of sodium iodide was begun on Dec. 3, 1943, on which day the bull weighed approximately 1,000 lb. Thirty Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Dec. 3, 6, 9, and 12, 1943. A total of 120 Gm. of the drug was administered during the nine-day period.

Observations.—Trichomonads were found on examinations subsequent to the seventh post-treatment week.

Result.—Treatment unsuccessful.

Second Treatment with Sodium Iodide and Potassium Iodide (at the farm).—Administration of iodine compounds was begun on March 15, 1944, on which day the bull weighed approximately 1,000 lb. Thirty Gm. of sodium iodide in 500 cc. of sterile distilled water were given intravenously on March 15 and 18, 1944. Ten Gm. of potassium iodide, in capsules, were given orally on each day from March 19 to April 17, 1944. A total of 60 Gm. of sodium iodide and 300 Gm. of potassium iodide was administered over the 34-day period.

Observations.—Trichomonads were found on examinations subsequent to the third post-treatment week.

Result.—Treatment unsuccessful.

Third Treatment with Sodium Iodide (at the farm).—Administration of sodium iodide intravenously was begun on Oct. 2, 1944, on which day the bull weighed approximately 1,000 lb. Forty-five Gm. of sodium iodide in 1 liter of sterile distilled water were given on Oct. 2 and 5, 1944, 25 Gm. on Oct. 8, 1944, in 500 cc. of water, and 45 Gm. in same way on Oct. 11, 14, and 17, 1944. A total of 450 Gm. of the drug was administered during the five-day period.

Observations.—Trichomonads were found on examinations subsequent to the fifth post-treatment week.

Result.—Treatment unsuccessful.

Fourth Treatment with Sodium Iodide plus Chloramine-T Douches (at Beltsville).—Administration of sodium iodide intravenously was begun on June 21, 1945, on which day the bull weighed 1,140 lb. Fifty Gm. of sodium iodide in 500 cc. of sterile distilled water were given on June 21, 23, 25, 27, and 29, 1945. A total of 250 Gm. of the drug was administered during the eight-day period. Administration of 0.25 per cent chloramine-T douches (2 liters) daily was begun on June 21, 1945, and continued through June 30, 1945. The sodium iodide was administered in the morning and the douche in the afternoon.

Observations.—(1) Preputial samples were examined at least once each week from June 21, 1945, until March 8, 1946. Trichomonads were not found at any examination subsequent to the beginning of treatment.

2) On Oct. 1 and 23, 1945, and Nov. 22, 1945, the bull served female 264. A pregnancy initiated by the later service terminated by abortion on Jan. 7, 1946. Bacteriologic examination of the embryo revealed only non-specific contaminants.* (At an earlier parturition, female 264 had suffered severe lacerations of the cervix and vagina; there was marked cervicitis and vaginitis *post partum*. At examination on Dec. 12, 1945, while pregnant, a moderate degree of cervicitis was evident.) On Nov. 8, 1945, the bull served female 233. Examination *per rectum* on March 8, 1946, revealed a normal pregnancy. On Nov. 27, 1945, and Jan. 11, 1946, he served female 241. Examination *per rectum* on March 8, 1946, revealed a normal pregnancy. On Dec. 25, 1945, and Jan 13, 1946, he served female 239. Examination *per rectum* on March 8, 1946, revealed a normal pregnancy.

3) Vaginal specimens from each of the 4 females were examined on each week day from the respective dates of first coitus until March 8, 1946. In no instances were trichomonads present.

Result.—Treatment successful.

• • •

Bull 207, a grade Guernsey, born May 19, 1940, was infected experimentally in the manner previously reported.[23] He was inoculated on Sept. 2, 5, 10, Nov. 24 and 25, 1941. Organisms were present in 128 of 160 samples examined between inoculation and Jan. 22, 1945. During this period, no attempts at treatment were made, but on May 15, 1943, one 40 Gm. dose of sodium iodide was given intravenously in 500 cc. of sterile distilled water. During the period of infection the bull transmitted infection through coitus to more than 10 females.

First Treatment with Sodium Iodide (at Beltsville).—Administration intravenously of

*Bacteriologic examinations were made by Dr. Chester A. Manthei, Pathological Division, Bureau of Animal Industry. His helpful co-operation, essential to this work, is gratefully acknowledged.

sodium iodide was begun on Jan. 22, 1945, on which day bull 207 weighed 1,450 lb. Sixty Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Jan. 22, 1945, and 50 Gm. on Jan. 24 and 26, 1945. Sixty Gm. were given on Feb. 12, 14, and 16, 1945. A total of 160 Gm. of the drug was administered during the four-day period of the first series of doses and after an interval of seventeen-days, 180 Gm. of the drug were administered during the second four-day period.

Observations.—Trichomonads were found on examinations subsequent to the second post-treatment week.

Result.—Treatment unsuccessful.

Second Treatment with Sodium Iodide plus Chloramine-T Douches (at Beltsville).—Administration of sodium iodide intravenously was begun on Oct. 18, 1945, on which day the bull weighed 1,450 lb. Sixty-five Gm. of sodium iodide in 500 cc. of sterile distilled water were given on Oct. 18, 20, 22, 24, and 26, 1945. A total of 325 Gm. of the drug was administered during the eight-day period. Administration of 0.25 per cent chloramine-T douches (2 liters) daily was begun on Oct. 18, 1945, and continued through Oct. 29, 1945. The sodium iodide was administered in the morning and the douche in the afternoon.

Observations.—Trichomonads were found on examinations subsequent to the second post-treatment week.

Result.—Treatment unsuccessful.

TOXICITY OF IODIDES

Sollmann[25] states that the immediate toxicity of iodides is low but that they disturb colloidal equilibrium. He further states that human beings do not evince reaction until a quantity of sodium iodide exceeding 10 Gm. has been given intravenously. Farquharson[26] recommended the use of sodium iodide intravenously at the rate of 1 oz. per 1,000 lb. of body weight, for the treatment of actinomycosis. He reported insignificant toxic effects from the treatment, except for abortion in pregnant subjects. Practically all discussions of iodine therapeusis in the literature warn of the irritation of the skin and mucous membranes (iodism) resulting from continued administration.

As reported in table 2, 8 bulls received iodides during the course of these experimental treatments of trichomoniasis. Eight additional animals (6 males and 2 females) were given the drugs primarily for pharmacologic and toxicologic studies. During or immediately following the intravenous administration of sodium iodide, occasional animals showed transient excitement, vertigo, or incoördination. One bull collapsed the day following an intravenously administered dose of sodium iodide, probably the result of overexertion from fighting. Recovery occurred within a few hours, but treatment was discontinued. Subsequently, no reaction occurred in this bull incident to another series of doses. Permanently harmful effects have not been observed in any animal involved in these experiments. One 590-lb. heifer, slaughtered forty-five days after having received, intravenously, 133 Gm. of sodium iodide in three doses over a six-day period, showed no detectable histologic abnormality of the kidneys, liver, or lungs. The dose given to this heifer was purposely large and the symptoms of iodism that developed were the most severe observed in these experiments. All the animals involved in the experiments manifested iodism in varying degrees. The earliest and most consistent symptoms were: staring appearance of the entire coat, formation of scurfy flakes about the neck, shoulders, perineum, and medial surface of the hind legs, and lost pliability of the skin so that stiff folds formed on movement. Animals experiencing more severe reactions showed epiphora, ptyalism, injection of all visible mucous membranes, tracheitis and bronchitis with coughing, mucopurulent nasal discharge, and loosening of the hair. A few animals showed irritation in the keratogenic areas of the claws. Temporary anorexia was observed in a few instances. The body weight during or after treatment was never materially altered.

Return to pretreatment appearance invariably occurred within a few weeks after the administration of iodides was discontinued.

According to some writers, iodine and iodine compounds have specific deleterious effect upon the gonads. Sollmann states

that "free iodine or loose iodine compounds (not KI) produce sterility in both sexes". As a result of the administration of iodides in these experiments, no evidence of permanently harmful effects upon the testicles (spermatogenesis) was observed. In all, ten semen samples were collected and examined during or soon after administration of iodides. During treatment, there were no consistent alterations of the semen picture, but significant numbers of spermatozoa with looped tails or reversed movement were found frequently in some samples. In no samples were there ever more than half nonmotile or abnormal forms. Subsequent to treatment, semen samples appeared normal. The results of post-treatment coitus as reported in the protocols are the most reliable indications of the lack of lasting effect upon fertility.

DISCUSSION

A rational approach to chemotherapy of bovine venereal trichomoniasis must take into consideration (1) the location of all *T. foetus* in the host and (2) the peculiar properties of trichomonadicidal agents and mixtures which will enable the active material to come into contact with all the organisms.

Investigation of the pathogenesis of this infection in bulls has revealed that the preputial cavity is the preferential site of *T. foetus*[17]. Hammond and Bartlett[24] found that the organisms usually occur in highest numbers on the portion of the glans penis not including the galea glandis, with relatively lower numbers scattered over the remainder of the penis and prepuce. Abelein[16], upon microscopic examination, found trichomonads deep in the folds and flask-like crypts of penile and preputial membranes.

Many workers[21, 27, 28, 29] have studied the action of numerous chemical agents on this pathogen *in vitro*. Their published results show that *T. foetus* is not a resistant form, being rapidly destroyed *in vitro* by most of the common disinfectants and antiseptics. *In vivo*, many of these same agents have failed.

Successful chemotherapy is evidently dependent primarily upon a trichomonadicidal agent reaching all the trichomonads. The observations made in the following described experiment give a probable explanation of the reason for the failure of aqueous solutions of simple trichomonadicides, when applied as douches, to cure bulls in which infection is confined to the prepuce.

The preputial cavity of an uninfected bull was thoroughly douched with an aqueous solution of methylene blue. Immediately following douching, the bull was killed and pieces of the genital membranes placed in Bethe's fixative. Sections prepared from these tissues were examined microscopically by Dr. Herman R. Seibold of the Pathological Division, Bureau of Animal Industry, who reported as follows: "There was some bluish coloration of the superficial cells of the epidermis in a majority of the sections . . . . . two deep crypts were observed into which there had been little, if any, penetration of the dye . . . . ." Consequently, it was concluded that all regions of the genital membrane were not reached by thorough douching of the prepuce with aqueous solutions.

Obviously, application of medicaments to the external genitalia and lower urethra cannot be expected to cure those bulls which harbor undisturbed trichomonads in their internal genitalia.

All agents used experimentally to treat bulls as reported in table 1 had evinced highly lethal properties in lesser concentrations on tests *in vitro*. In addition, trichomonadicidal agents B and D (table 1) both possessed remarkable properties as detergents and surface tension depressants and should, therefore, theoretically, favor ultimate penetration into the less accessible regions of the membranes. Ointments 1 and 2 (table 1) were both water soluble and readily miscible with the secretions of genital membranes and should, theoretically, be excellent vehicles for ac-

tive agents. All proved ineffective under the conditions of these experiments.

The series of experimental treatments shown in table 1, with the exception of the one trial with the trypaflavine ointment recommended by Abelein and Swangard, were completed in 1943. These experiments preceded those reported in table 2. Since no indication of therapeutic value of the former procedures had been revealed, the failures being constant, it was decided at that time to abandon the line of investigation utilizing topical application of trichomonadicides. Trypaflavine had shown no unusual efficacy on tests *in vitro* and a similar trichomonadicide having at least equal efficacy, combined with an ointment base having similar properties to the one used by Abelein and Swangard, showed no merit in tests performed by the writer. In May, 1945, sufficient trypaflavine ointment to treat 1 bull was obtained from Dr. Swangard. Treatment was carried out in accordance with the procedure described, but the infection continued. Further investigations of this ointment, under controlled experimental conditions, are necessary before the usefulness of this specific chemotherapeutic agent can be properly evaluated.

When experimental procedures designed to cure trichomoniasis in bulls are considered theoretically, an approach in which all tissues are reached seems to be ideal. Iodides, because of certain of their pharmacologic characteristics, seemed to offer such theoretical possibilities. Administration orally or intravenously of iodides is known to be followed by the appearance of iodine in some form in practically all secretions and excretions of the body[30]. Since iodine is known to appear in considerable concentrations in the bronchial mucus[31], it was hypothesized that iodine might be secreted by the glandular structures of the male genitalia in sufficient concentration to destroy trichomonads.

Subsequent chemical tests demonstrated that iodine does appear in appreciable concentration in the prepuce, presumably secreted on the membranes following administration of large doses of iodides.

Both sodium and potassium iodides are known to exhibit only slight trichomonadicidal action on tests *in vitro*. Whether the iodine is eliminated in these or other chemical forms is not yet known, but it is obvious that the compound is not immediately toxic for *T. foetus*. One female treated during infection was not cured and samples of genital mucus containing large numbers of living *T. foetus* were tested chemically and found positive for iodine.

The experiments herein reported, in which iodine therapy in trichomoniasis was investigated, are to be considered as pilot experiments. The results are to be considered preliminary, and this report, itself, is to be considered only a progress report of research completed to date. These incomplete investigations are presented at this time because several years must necessarily elapse before significant additional observations can be accumulated in this laboratory. The therapeutic value of potassium iodide, sodium iodide, and combination of the two, in treatment of trichomoniasis is still uncertain. Cures have resulted from these treatments, but the number of individuals requiring more than one treatment before being cured, and the number of individuals that have remained infected to date despite several treatments, indicate that there are as yet unknown factors that limit the efficacy of these treatments. These factors must be recognized and overcome before these procedures can be considered for practical application. Further investigations probably will reveal more advantageous regimens of iodide administration. At present, three to five doses of sodium iodide given intravenously at 48-hour intervals at the rate of 30 to 50 Gm. per 1,000 lb. of body weight, is considered the most efficacious and practical regimen in experimental treatment. Douching concomitantly with an oxidizing agent, chloramine-T, evidently has no value.

SUMMARY AND CONCLUSIONS

1) Three bulls infected with *Trichomonas foetus* received 12 experimental treatments or series of treatments with six active trichomonadicidal agents (sodium hypochlorite, sodium perborate, Lugol's solution, sodium dioctyl sulfo-succinate, sodium ethyl mercuri thiosalicylate, alkyl dimethyl benzyl ammonium chlorides) applied as preputial douches or ointments. No bulls were cured.

2) It is concluded that topical application of the above trichomonadicides as employed in these experiments shows no promise in treatment of trichomonad-infected bulls.

3) One bull was treated unsuccessfully with trypaflavine ointment in accordance with the procedure of Abelein and Swangard.

4) Eight bulls infected with *T. foetus*, 2 of which were experimentally reinfected subsequent to being cured (a total of ten infections), received 21 courses of treatment with potassium iodide orally and/or sodium iodide, intravenously. In six instances, these treatments were supplemented by preputial douches with an oxidizing agent (chloramine-T). Six infections were cured and four were not.

5) It is concluded on the basis of the results obtained from experimental iodide treatments that iodides show considerable value as chemotherapeutic agents in trichomoniasis in bulls. No evidence was found to indicate that the efficacy of iodide treatment was enhanced by concomitant douching of the prepuce with an oxidizing agent.

References

[1]Cameron, Hugh S., Fincher, M. G., and Gilman, Herbert L.: Trichomonas Infection in Cattle. Cornell Vet., *23*, (1933): 297-303.

[2]Abelein, R.: Bekämpfung der Trichomonadenseuche. Deutsche Tierärztl. Wchnschr., *42*, (1934): 449-453.

[3]Brauer, W.: Untersuchungen über die Verbreitung und Bekämpfung der Trichomonadenerkrankung des Rindes. Inaug. Diss. (Giessen), (1935): 66 pp.

[4]Sontgen, K.: Die Zuchtausfallschaden im Regierungsbezirk Wiesbaden mit besonderer Berücksichtigung der Trichomonadeninfektion. Tierärztl. Rundschau, *41*, (1935): 319-323.

[5]Küst, Dietrich: Seuchenartige Aborte und Unfruchtbarkeit des Rindes mit besonderer Berücksichtigung der Trichomonadenseuche. Züchtungskunde, *10*, (1935): 81-90.

[6]Küst, Dietrich: Die Trichomonadensterilität des Rindes und ihre Bekämpfung. Deutsche Tierärztl. Wchnschr., *44*, (1936): 177-181.

[7]Möller, Edgar: Beobachtungen über Trichomonadenseuche des Rindes. Tierärztl. Rundschau, *42*, (1936): 444-448.

[8]Benesch, F., and Wurst, R.: Bericht über einen schweren Trichomonadenbefall bein Rindern auf einem Gutshof in Niederösterreich. Wiener Tierärztl. Monatschr., *23*, (1936): 481-487.

[9]Gould, G. N.: Trichomonas Disease of Cattle. Description of the Outbreak. Vet. Rec., *49*, (1937): 247-253.

[10]Schmidt, H.: Trichomoniasis or Trichomonad Abortion in Cattle. J. A. V. M. A., *90*, (1937): 608-618.

[11]Schmidt, H., Turk, R., and Shepardson, C.: Trichomonad Abortion in Cattle. Texas Agric. Sta. Bull., *50*, (1937): 14-15.

[12]Schmidt, H., Turk, R., and Shepardson, C.: Trichomonad Abortion in Cattle. Texas Agric. Sta. Bull., *51*, (1938): 13.

[13]Schmidt, H., Turk, R., and Shepardson, C.: Trichomonad Abortion in Cattle. Texas Agric. Sta. Bull., *52*, (1939): 13.

[14]Schmidt, H., Turk, R., and Shepardson, C.: Trichomonad Abortion in Cattle. Texas Agric. Sta. Bull., *53*, (1940): 11.

[15]Schmidt, H., Turk, R., and Shepardson, C.: Trichomonad Abortion in Cattle. Texas Agric. Sta. Bull., *54*, (1941): 92.

[16]Abelein, R.: Behandlung von Bullen mit Trichomonaden. Deutsche Tierärztl. Wchnschr., *46*, (1938): 721-724.

[17]Abelein, R.: Trichomonadenseuche beim Bullen und ihre Behandlung. Berl. Münch. Tierärzl. Wchnschr., July 25, (1941): 357-362; Aug. 1, (1941): 370-377.

[18]Swangard, W. M.: Control of Trichomoniasis in Cattle. Vet. J., *94*, (1938): 446-453.

[19]Swangard, W. M.: Trichomoniasis in Cattle: Biological Studies and System of Control. J. A. V. M. A., *95*, (1939): 146-151.

[20]Swangard, W. M.: A Further Note on Diagnosis and Treatment of Bovine Trichomonas Infection. J. A. V. M. A., *99*, (1941): 404-406.

[21]Kerr, W. R.: Trichomoniasis in Bulls. II. Vet. J., *99*, (1943): 4-8.

[22]Wright, J. G.: Observations on the Clinical Aspects of Reproductive Disorders in Cattle. Vet. Rec., *57*, (1945): 316-320.

[23]Hammond, Datus M., and Bartlett, David, E.: Establishment of Infection with *Trichomonas foetus* in Bulls by Experimental Exposure. Am. J. Vet. Res., *4*, (1943): 61-65.

[24]Hammond, Datus M., and Bartlett, David, E.: The Distribution of *Trichomonas foetus* in the Preputial Cavity of Infected Bulls. Am. J. Vet. Res., *4*, (1943): 143-149.

[25]Sollmann, Torald: A Manual of Pharmacology and Its Application to Therapeutics and Toxicity. Sixth Ed., 1943.

[26]Farquharson, James: Intravenous Use of Sodium Iodide in Actinomycosis. J. A. V. M. A., *91*, (1937): 551-554.

[27]Senna, Virgilio: Il Trichomonas utero vaginalis vitulae e la sua resistenza agli agente fisici e chimici. Clin. Vet., *59*, (1936): 301-312.

[28]Mussill, J.: Untersuchungen über die Widerstandsfähigkeit der Rinder-Trichomonaden gegen physikalische und chemische Einflüsse. Wiener Tierärztl. Monatschr., *25*, (1937): 104-109.

[29]Morgan, Banner Bill: Bovine Trichomoniasis. 1944.

[30]Milks, Howard Jay: Practical Veterinary Pharmacology, Materia Medica, and Therapeutics. Third Ed., 1936.

[31]Tuft, Louis, and Levin, Nathaniel M.: Studies of the Expectorant Action of Iodides., A. J. M. Sci., *203*, (1942): 717-722.

Bacteriopathology of Infectious Bovine Pyelonephritis

E. S. FEENSTRA, D.V.M., M.S., and F. THORP, Jr., D.V.M., M.S., Ph.D.

East Lansing, Michigan

THE LOCATION of the causative agent in relation to the resulting lesion is an interesting and important factor in the study of disease and an aid in understanding the nature of the lesion and its effect on the animal body. It is our intent here to review the literature, study, and discuss the bacteriopathology of infectious bovine pyelonephritis, the cause of which is reputed to be *Corynebacterium renale.*

REVIEW OF LITERATURE

Dammann[1] found bacteria in the urinary tubules. Guillebeau[2] found slender nonmotile bacilli in renal abscesses. Höflich[3] mentioned, "On one occasion only, in some sections stained with hematoxylin, the organisms were seen in the glomeruli while felted masses were constantly met with in the terminal portions of the straight tubes and in the epithelium of the pelvis and ureter." Ernst[4] described the condition in the kidney. Speaking first of the small elongated abscesses around the vessels in the medulla, he stated, "Gram's stain reveals masses of bacteria scattered irregularly through the abscesses; these masses surround the malpighian corpuscles like a cap. The bacteria are collected into clumps and cylinders in the convoluted tubes and in the loops of Henle, becoming less frequent in the subcortical zone. Groups of bacteria are rare in the thrombosed vessels. The straight tubes, which are packed with bacteria eliminated from the labyrinth, give the medullary zone a striated appearance. The papillary tubes appear free, but their mucous membrane is infiltrated with leucocytes." Describing a later stage, that of papillary necrosis, he stated, "Disintegration of the collecting tubes of the papilla, which are crammed with bacteria, débris, and leucocytes, occurs."

Jones and Little[5] stated that the organisms were present in the infected kidney pelves. Also, bacteria were found in large numbers in the necrotic débris on the mucosa of the bladder. From these data and the fact that the bacteria were never found in the deeper structures, they deduce that the organism is not very invasive. In 1930, the same authors[6] noted densely packed masses of diphtheroids in the large tubules lying close to the papilla

Fig. 1—Bladder mucosa showing general architecture of tissue alteration. Cellular débris, necrotic tissue, and zone of chronic inflammation. Gram-Weigert stain. x95.

in the kidney. Thorp[7] *et al.* mention the presence of *C. renale* in tubular casts in the kidney, in necrotic tissue of the renal papillae,

Published with the permission of the Director of the Experiment Station as Journal Article 792 n.s.

From the Animal Pathology Section, Michigan Agricultural Experiment Station, East Lansing, Mich.

and along the mucosal surface of the ureters and bladder.

MATERIAL AND METHODS

Sections of kidney, ureter, and bladder from cases of bovine pyelonephritis were stained by the Gram-Weigert method for staining bacteria in sections and examined to determine the location of the bacteria and their relationship to the lesion produced.

RESULTS

There was much variation between cases. In some instances, the bacteria seemed to be only superficially located in the cellular débris of the kidney calyces and in similar material adherent to the mucosa of the ureters and bladder. In these cases, there was local necrosis and an areal increase in the number of lymphocytes and the connective tissue elements. There was no direct invasion of the living tissues as far as could be determined in these cases. In other instances, the bacteria were located superficially as in those cases mentioned above but also were found in scattered foci throughout the kidney cortex and in the thickened capsule. Their presence was always attended by necrosis and inflammatory reactions of a chronic type — infiltration of tissues with lymphocytes and a few

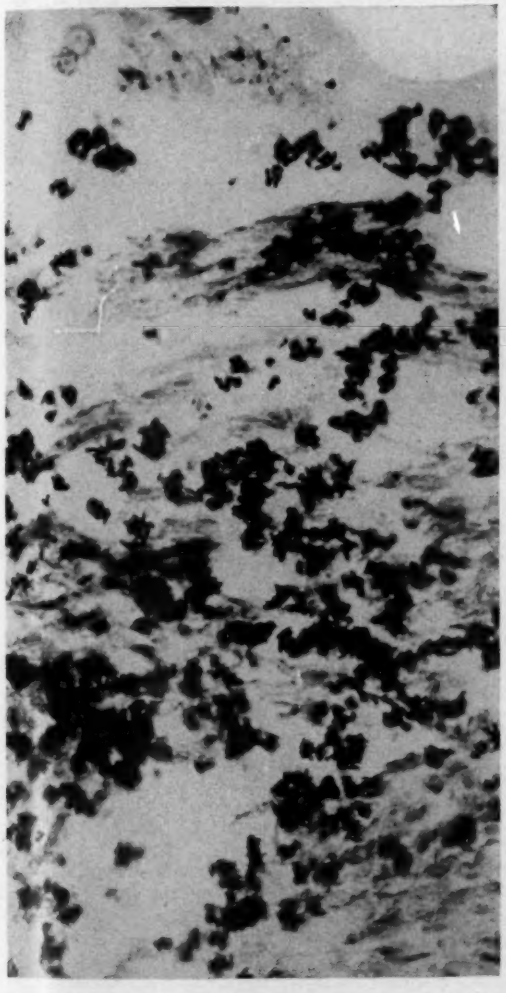


Fig. 2—Bacteria in cellular débris in fig. 1. Gram-Weigert stain. x770.

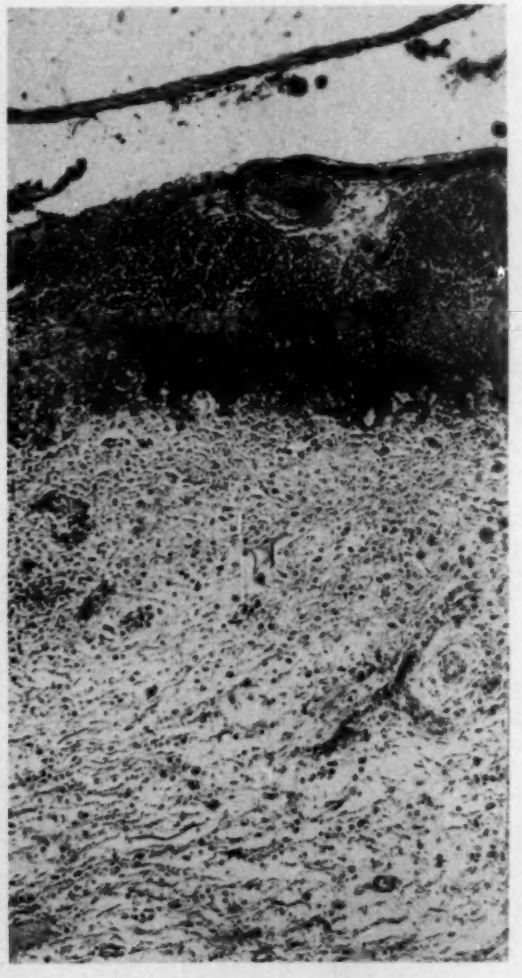


Fig. 3—Ureter. General architecture of lesion. Gram-Weigert stain. x95.

mononuclear phagocytes plus an increase of the interstitial connective tissue. These foci varied from very small ones, in which only a few tubules were found to contain bacteria which were causing necrosis of the epithelial cells, to fairly large cortical abscesses which contained much dead tissue and large numbers of bacteria and were surrounded by the chronic inflammatory reaction already mentioned. Local necrosis surrounding each clump of bacteria was a constant feature. The clumps of bacteria varied in size from several short rods to large, amorphous, dark-staining masses, along the edges of which a few bacteria were visible, enabling recognition of the material.

DISCUSSION

The seeming difference in virulence or invasiveness of the diphtheroids in the various cases studied is in keeping with the variation between diphtheroids, which seems to be one of their characteristics. The difference in susceptibility of the infected animals should also be considered.

Possibly the location of the lesions and of the etiologic agent is a factor in explaining the course of the disease in different cases. Some cases are of a chronic nature

Fig. 4—Bacteria in ureteral lesion similar to that shown in fig. 3. Gram-Weigert stain. x550.

Fig. 5—Inner medulla of kidney. General architecture of lesion. Gram-Weigert stain. x82.

while others are fulminating and terminate soon. The more mild and drawn out type of case may be associated with the superficial, slowly advancing type of lesion in which the bacteria are in the calycal cellular débris and seem to cause the medulla to be eroded slowly. In later stages, lesions may be found throughout the kidney. The fulminating type of case may be associated with the more severe lesions in the kidney with scattered abscesses throughout.

One of the more striking observations is that, except in case of the lesions in the kidney tubule, the bacteria seem rarely to be in contact with living tissue. This is especially apparent in the kidney calyces, the ureter, and the bladder. The bacteria are found in the tissue débris next to a necrotic area in the mucosa which is bordered on the opposite side by a chronic inflammatory zone. Observation of this type of lesion and the fact that the type species of the same genus produces a powerful toxin has stimulated the search for a toxin produced by the diphtheroids isolated from cases of pyelonephritis. So far, no one has reported demonstration of any such toxin. One may only conjecture that the necrosis is the result of an endotoxin which is not apparent in culture filtrates.

The inherent difficulties in finding single or a few bacteria in tissue sections are realized. It is possible that more exacting culturing techniques would aid in further establishing the location of bacteria at least in relation to the medulla and cortex of the kidney.

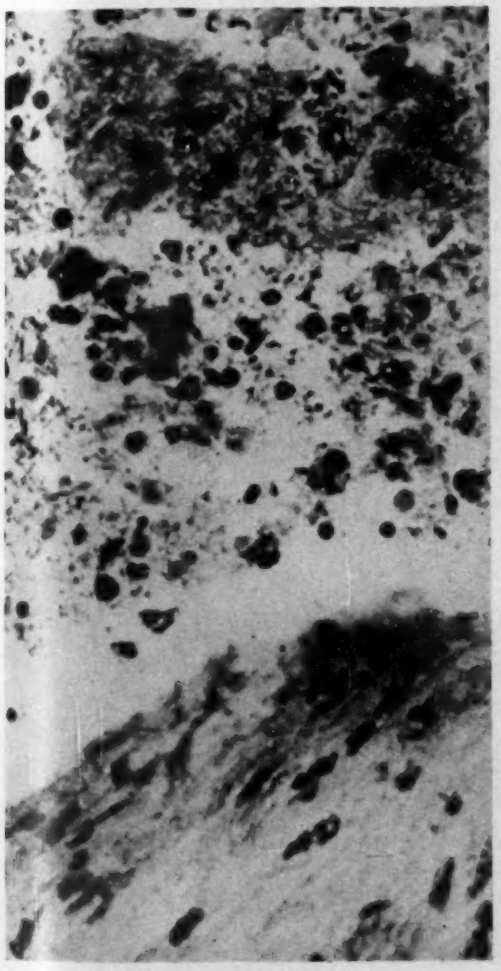


Fig. 6—Bacteria in cellular débris in lesion similar to that in fig. 5. Gram-Weigert stain. x770.

Fig. 7—Cortex of kidney. Masses of bacteria in tubules. Necrosis of epithelium. Foci of lymphocytes. Gram-Weigert stain. x95.

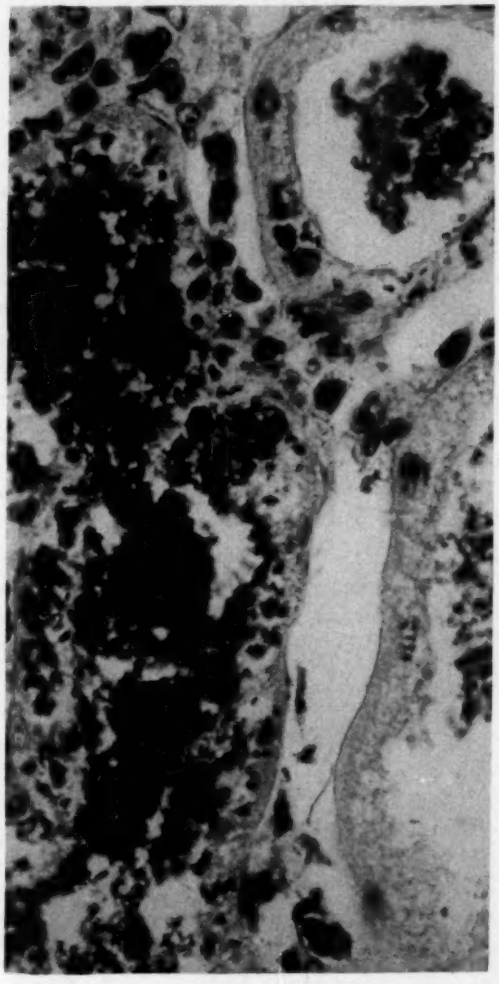


Fig. 8—Bacteria in kidney tubule. Gram-Weigert stain. x770.

SUMMARY

The location of the bacteria varied in different cases. In the milder cases, the microörganisms were present seemingly only in the cellular débris of the kidney calyces, the ureter, and bladder mucosa. In the more severe cases, the microörganisms also may be found in foci scattered throughout the kidney. Bacteria may be found in the tubules and necrotic tubular epithelium. In the characteristic lesion, the diphtheroids were in cellular débris which was flanked by a zone of necrosis and, outside of that, a zone showing chronic inflammatory changes.

References

[1]Dammann: Ztschr. f. Tiermedizin (1877). Cited by Ritzenthaler, M.: The Pathology of Bacillary Pyelonephritis in Cattle. J. Comp. Path. and Therap., *23*, (1910): 33-50.

[2]Guillebeau: Schweiz. Arch. f. Tierheilk., *30*, (1888): 269. Cited by Ritzenthaler, M.: The Pathology of Bacillary Pyelonephritis in Cattle. J. Comp. Path. and Therap., *23*, (1910): 33-50.

[3]Höflich: Monatsch. f. Prakt. Tierheilk., *2*, (1891): 337. Cited by Ritzenthaler, M.: The Pathology of Bacillary Pyelonephritis in Cattle. J. Comp. Path. and Therap., *23*, (1910): 33-50.

[4]Ernst: Centralbl. f. Bakt., *39*, (1905): 549-660: *40*, (1905): 79. Cited by Ritzenthaler, M.: The Pathology of Bacillary Pyelonephritis in Cattle. J. Comp. Path. and Therap., *23*, (1910): 33-50.

[5]Jones, F. S., and Little, R. B.: Specific Infectious Cystitis and Pyelonephritis of Cows. J. Exptl. Med., *42*, (1925): 593-607.

[6]Jones, F. S., and Little, R. B.: A Contribution to the Epidemiology of Specific Infectious Cystitis and Pyelonephritis of Cows. J. Exptl. Med., *51*, (1930): 909-920.

[7]Thorp, F., Jr., Langham, R. F., Clark, C. F., and Doll, E. R.: The Pathology of Bovine Pyelonephritis. Am. J. Vet. Res., *4*, (1943): 240-249.

The Histopathology of a Focal Hepatitis and of its Termination ("Sawdust" and "Telang" Liver) in Cattle

ROBERT GETTY, D.V.M., M.S.
Ames, Iowa

THE MEAT-PACKING industry has sustained a heavy loss for years because of the condemnation, upon federal inspection, of bovine livers affected with telangiectasis and the so-called "sawdust" condition. Due to scientific advances, the monetary value and nutritional significance of the bovine liver have materially increased in recent years. The economic importance of the loss of this food product is apparent in the reports of the National Live Stock Loss Prevention Board[1]. During the years 1941 to 1944, inclusive, a total of 910,962 beef livers were condemned for telangiectasis and 426,367 as "sawdust" livers by the federal meat inspectors. In 1944, a total of 12,411,374 cattle were slaughtered under federal inspection; from this number, 236,324 livers were condemned for telangiectasis and 117,871 for "sawdust" condition. When one considers that the average weight of the bovine liver is 10 to 12 lb. (Sisson and Grossman[2]), and the wholesale value is approximately 25 to 30 cents per pound, it is evident that the annual loss exceeds one million dollars.

The term telangiectasis, as defined in Stedman's Medical Dictionary, means "dilatation of the small or terminal blood vessels of a part." However, a review of the literature reveals that a variety of terms have been applied to this condition, among which the following may be mentioned: cavernous angioma (McFadyean)[3], hemorrhagic infarct (Stubbe)[4], cavernous angiomata (Stockman)[5], spotted capillarectasis (Stroh)[6], capillary telangiectasis (Kitt)[7], telangiectasis (Jaeger)[8], hemorrhagic spotty livers (Chaussé)[9], and cavernous hemangioma. The variety of terms used to describe this condition emphasizes the confusion existing as to its nature and cause. The term "sawdust", as defined by Edelmann[10] and used by the meat-packing industry, is applied to those livers exhibiting yellowish white areas the size of a small particle of sawdust, but descriptive literature is deficient regarding these livers.

With the economic and nutritional factors in mind, studies have been conducted since February, 1943, on telangiectatic and "sawdust" bovine livers, in an attempt to establish a definite macroscopic and microscopic picture of the condition. An attempt was also made to determine the percentage of distribution and the incidence of telangiectatic and "sawdust" livers in relation to sex, age, and condition of the animal. The disposition of the carcass and other concurrent lesions, including parasitism, were also noted.

METHOD OF PROCEDURE

A) Obtaining Liver Samples.—The livers used in this study were obtained from both beef and dairy cattle slaughtered at the plants of Wilson and Company, Omaha, Neb., Cedar Rapids, Iowa, and Chicago, Ill. Both superficial and deep samples, usually several centimeters square, were taken from the peripheral and central portions of the livers. The specimens were fixed in absolute alcohol and in 10 per cent solution of formalin (equal to 4 per cent of formaldehyde), and returned to the laboratory at Iowa State College for embedding, sectioning, and mounting. Glycogen deposition was determined in those samples placed in absolute alcohol, while fat stains and various routine standard and modified stains were made from the samples placed in 10 per cent formalin.

B) Routine Observations.—Because the

From the Department of Veterinary Pathology, Veterinary Division, Iowa State College. For the entire work and additional photomicrographs and references see "The Histopathology of a Focal Hepatitis and of its Termination ("Sawdust" and "Telang" Liver) in Cattle," Master's Thesis, Library, Iowa State College (1945). Supported in part by a grant from the National Live Stock and Meat Board.

The author is assistant professor, Department of Veterinary Anatomy, Iowa State College, Ames.

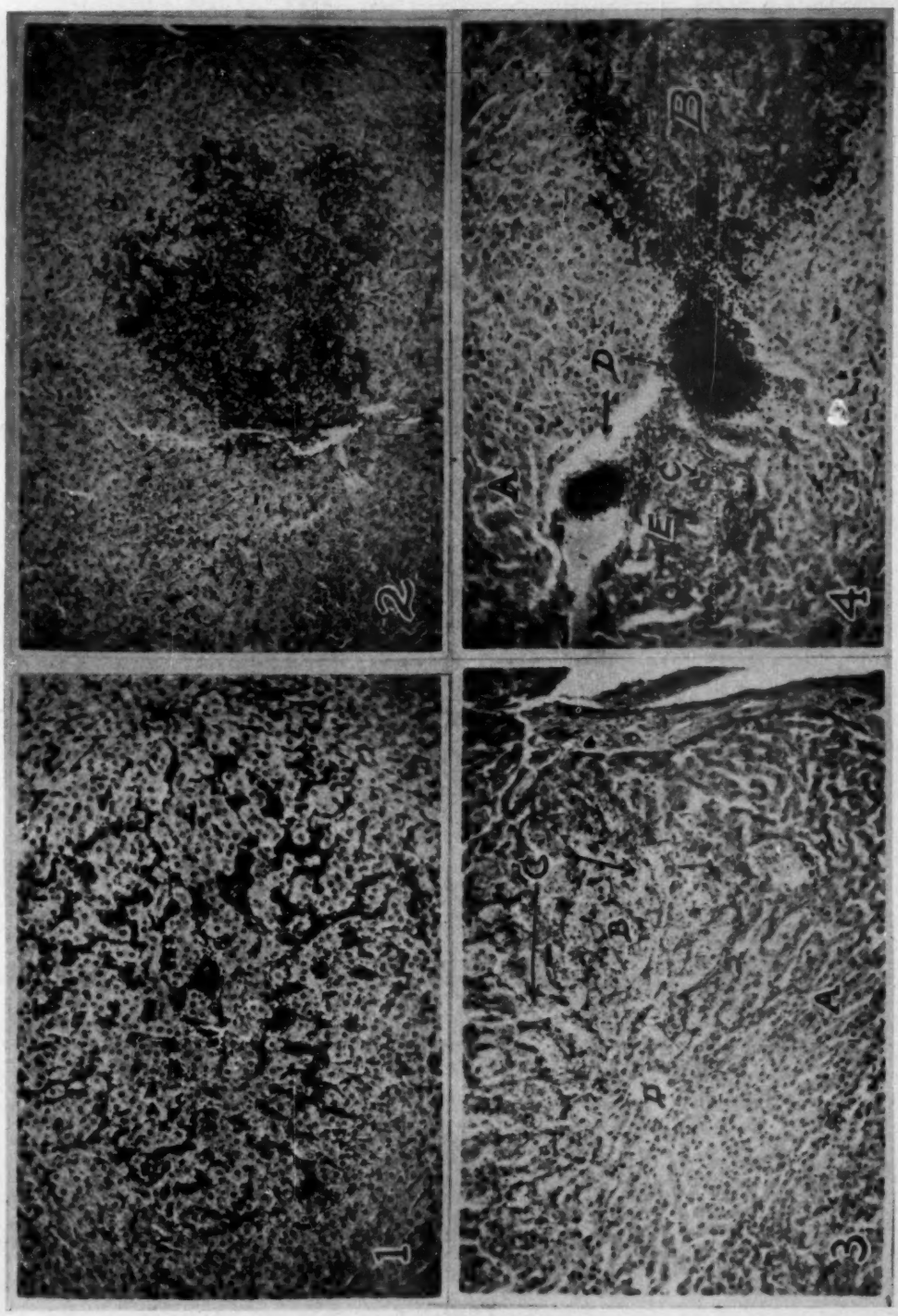


Fig. 1—Photomicrograph of an hepatic area illustrating the first stage of the described focal hepatitis. Note the distended sinusoids engorged with blood. x 130, Specimen 27.

Fig. 2—The macroscopic, purplish, pin-point telangiectatic areas exhibit a microscopic picture similar to that seen in this figure. x 130. Specimen 87.

Fig. 3—Note the distended sinusoids with accompanying hyperemia in one half of hepatic lobule; hepatic parenchyma apparently normal on opposite side of central vein. x 130. Specimen 53. (A) Normal hepatic cells; (B) enlarged sinusoids; (C) area of hyperemia; (D) central vein.

Fig. 4—Note the leucocytic infiltration of the periportal necrotic focus. x 130. Specimen 26. (A) Normal hepatic cells; (B) necrotic focus;

material was obtained from the killing floor, the following observations could be made: the approximate age, the sex, breed, general condition of the carcass, other concurrent lesions including parasitism, and disposition of carcass. The livers were examined grossly as to size, weight, shape, color, consistency, and odor, and incised for evidence of hypertrophy, fascioliasis, or any other visible abnormality.

C) Histopathologic Procedures.—A modification of the techniques described by Mallory[11] and by Salthouse[12] was used in order to examine the tissues for fat. Representative samples of the various conditions were embedded in a glycerine-gelatine solution and placed in an incubator at 56 C. for three to four hours. Upon removal and solidification, a block was cut, trimmed, and frozen sections were made from it. The sections were stained with scarlet red following the technique described by Mallory. A rapid fat stain using oil red "O" was also used following the technique of Proescher[13], as well as the nile blue sulfate stain introduced by Smith[14].

The remaining duplicate samples, fixed in 10 per cent formalin, were dehydrated and cleared by transfer through three changes of dioxane (Guyer)[15]. The tissues were impregnated with paraffin, using Altmann's embedding mixture with three changes of paraffin kept at a constant temperature of 56 C. Two tissue blocks were taken from each representative liver sample and marked "A" and "B", respectively; the thickness of the sections was governed by the condition of the paraffin and the ease of sectioning. Five slides were made from each block so that the following stain techniques, as described by Mallory, could be routinely employed. The sections were stained with Harris' hematoxylin and eosin, Heidenhain's hematoxylin, Weigert's elastic tissue stain, and Van Gieson's connective tissue stain.

Some of the tissues, fixed in absolute alcohol, were embedded in celloidin and stained with Best's carmine following the embedding and staining methods of Mallory[11]; others were embedded and stained for glycogen following the technique described by Bensley[16] for paraffin embedding and selective staining for glycogen.

RESULTS

Macroscopic Findings.—Macroscopically, the typical telangiectatic liver appeared normal in size, weight, and shape. The lesions, which were purplish black to reddish blue, varied greatly. Some of the lesions were but pinpoint in size, others 1 cm. or more. They possessed rather definite boundaries which marked the involved area from the remaining hepatic parenchyma. However, the very small, purplish, pinpoint areas could be easily overlooked unless careful observation was made. The larger lesions, which may be sunken, could not only be seen but also palpated on the surface. Upon incision, the lesions exhibited a meshlike or netlike structure which was filled with blood. They were sometimes confined to a very small area of the liver, or were often disseminated throughout the entire organ. The remaining hepatic parenchyma appeared normal. No cirrhosis, hypertrophy, or evidence of fascioliasis was noted.

Macroscopically, the "sawdust" livers appeared normal in size, weight, and shape. The lesions were pinkish white to yellow-grey and variable in distribution. They were distributed throughout the entire liver or were found only in irregularly scattered areas. The noninvolved areas appeared normal in color, consistency, and odor. Upon incision, the cut surfaces approximated one another, indicating no hypertrophy. The surface of the organ was smooth and no cirrhosis was evident on palpation.

Telangiectatic and 'sawdust" lesions were frequently noted in the same liver. These lesions, which were variable in extent and distribution, exhibited the combined appearance of the previously described telangiectatic and "sawdust" livers.

Microscopic Findings.—The following results were representive of those obtained from the examination of several thousand sections made from 82 liver specimens. The first phase in the observed focal hepatitis was one of distended sinusoids engorged with blood (fig. 1). Frequently, the involved areas could not be observed grossly; microscopically, they had the appearance of an area in the very first stages of a local inflammation. The pinpoint telangiectatic lesions were at first confined to a single hepatic lobule (fig. 2, 3). No leucocytosis was evident at this early stage. Pressure

Fig. 3—Note the distended sinusoids with accompanying hyperemia in one half of hepatic lobule; hepatic parenchyma apparently normal on opposite side of central vein. x 130. Specimen 53. (A) Normal hepatic cells; (B) enlarged sinusoids; (C) area of hyperemia; (D) central vein.

Fig. 4—Note the leucocytic infiltration of the periportal necrotic focus. x 130. Specimen 24. (A) Normal hepatic cells; (B) necrotic focus.

TABLE I—Sex, Age, Condition of Carcass, and Classification of 82 Liver Specimens

Specimen number	Sex	Age	Condition of carcass	Macroscopic classification	Microscopic classification
1	F	18-24 mo.	Fancy	Normal	Normal
2	F	18-24 mo.	Prime	Sawdust	Sawdust (sl.)
3	F	8-10 yr.	Good	Telang	Telang (sl.)
4	M	18-24 mo.	Prime	Sawdust	Sawdust (sl.)
5	M	18-24 mo.	Prime	Telang	Telang (sl.) Sawdust (mod.)
6	M	18-24 mo.	Choice	Telang Sawdust	Sawdust (sl.)
7	M	18-24 mo.	Choice	Normal	Normal
8	M	18-24 mo.	Choice	Sawdust	Sawdust (sl.)
11	M	18-24 mo.	Choice	Sawdust	Sawdust (sl.)
12	M	18-24 mo.	Prime	Sawdust	Sawdust (sl.)
13	M	18-24 mo.	Prime	Telang	Telang (sl.) Sawdust (ext.)
14	M	18-24 mo.	Prime	Sawdust	Sawdust
15	M	18-24 mo.	Prime	Sawdust	Sawdust
16	M	18-24 mo.	Choice	Normal	Normal
17	F	18-24 mo.	Choice	Normal	Normal
18	F	8-10 yr.	Good	Telang	Telang (sl.)
19	M	18-24 mo.	Choice	Telang Sawdust	Telang (sl.) Sawdust (ext.)
21	F	18-24 mo.	Fancy	Normal	Sawdust Telang
22	F	18-24 mo.	Good	Sawdust (ext.)	Sawdust (mod.)
23	F	12 yr.	Canner	Telang (ext.)	Telang (ext.)
24	F	18-24 mo.	Choice	Sawdust (ext.)	Sawdust (ext.) Telang (sl.)
25	F	18-24 mo.	Choice	Sawdust (ext.)	Sawdust Telang
26	F	18-24 mo.	Prime	Sawdust (mod.)	Sawdust (mod.)
27	F	18-24 mo.	Prime	Sawdust (mod.) Telang (mod.)	Sawdust (ext.) Telang (sl.)
28	F	6 mo.	Prime	Sawdust (sl.)	Sawdust (v.sl.) Telang (sl.)
29	M	2 yr.	Prime	Telang (v.sl.)	Sawdust (mod.) Telang (v.sl.)
30	F	12-15 yr.	Canner	Telang (ext.)	Telang (ext.)
31	F	2 yr.	Prime	Sawdust (ext.)	Sawdust (ext.) Telang (sl.)
41	F	18-24 mo.	Fancy	Normal	Sawdust (sl.) Telang (sl.)
42	F	18-24 mo.	Prime	Sawdust (ext.)	Sawdust (sl.)
43	F	12 yr.	Canner	Telang (ext.)	Telang (mod.)
44	F	18-24 mo.	Choice	Sawdust (ext.)	Sawdust (ext.) Telang (sl.)
45	F	18-24 mo.	Choice	Sawdust (ext.)	Sawdust Telang
46	F	18-24 mo.	Prime	Sawdust (mod.)	Sawdust (mod.)
47	F	18-24 mo.	Prime	Sawdust (mod.) Te'ang (mod.)	Sawdust (ext.) Telang (sl.)
48	F	6 mo.	Prime	Sawdust (sl.)	Sawdust (v.sl.) Telang (sl.)
49	M	2 yr.	Prime	Telang (v.sl.)	Sawdust (mod.) Teland (v.sl.)
50	F	12-15 yr.	Canner	Telang (ext.)	Telang (ext.)
51	F	2 yr.	Prime	Sawdust (v. ext.)	Sawdust (ext.) Telang (sl.)
52	?	yearling	Prime	Sawdust	Sawdust (mod.) Telang (sl.)

*M represents bull or steer; F represents cow or heifer.
See explanatory paragraph, p. 442, for key to abbreviations.

TABLE I (Continued)—Sex, Age, Condition of Carcass, and Classification of 82 Liver Specimens

Specimen number	Sex	Age	Condition of carcass	Macroscopic classification	Microscopic classification
53	?	yearling	Prime	Telang (ext.)	Telang (ext.) Sawdust (ext.)
54	F	12-15 yr.	Canner	Telang	Telang (mod.)
55	F	12-15 yr.	Canner	Sawdust (sl.)	Sawdust (sl.)
56	M	old bull	Canner	Normal	Telang (v.sl.)
57	F	12-15 yr.	Canner	Telang (sl.)	Telang (mod.)
58	?	yearling	Choice	Sawdust	Sawdust (sl.)
59	?	yearling	Prime	Sawdust	Sawdust (mod.) Telang (mod.)
60	F	10-12 yr.	Good	Normal	Normal
61	?	yearling	Prime	Sawdust	Sawdust (sl.)
62	?	yearling	Prime	Sawdust	Sawdust (sl.)
63	?	yearling	Choice	Telang (ext.)	Telang (ext.)
64	F	12-15 yr.	Canner	Telang	Telang (ext.) Sawdust (sl.)
65	M	old bull	Canner	Normal	Areas of degeneration noted
66	M	old bull	Canner	Normal	Normal
67	M	18-24 mo.	Prime	Telang	Sawdust (ext.) Telang (mod.)
68	F	12-15 yr.	Good	Telang	Telang (ext.)
69	M	young bull	Prime	Sawdust (sl.)	Sawdust (sl.) Telang (early)
70	M	18-24 mo.	Prime	Sawdust	Sawdust Telang
71	F	12-15 yr.	Canner	Telang (sl.) Sawdust (sl.)	Sawdust (sl.) Telang (v.sl.)
72	?	yearling	Choice	Telang (ext.)	Telang (ext.) Sawdust (sl.)
73	?	yearling	Choice	Sawdust	Sawdust (ext.)
74	F	10-12 yr.	Utility	Telang	Telang (ext.) Sawdust (v.sl.)
75	F	8 yr.	Good	Sawdust	Sawdust (mod.) Telang (sl.)
76	?	yearling	Prime	Normal	Normal
77	?	yearling	Prime	Sawdust	Sawdust (mod.)
78	F	6-8 yr.	Good	Telang	Telang (mod.)
79	?	yearling	Prime	Normal	Telang (sl.)
80	?	yearling	Choice	Sawdust	Area not sectioned
81	F	8-10 yr.	Good	Telang Sawdust	Telang (mod.) Sawdust (mod.)
82	F	10-12 yr.	Canner	Normal	Normal
83	F	10-12 yr.	Canner	Telang Sawdust	Telang (ext.) Sawdust (sl.)
84	F	10-12 yr.	Canner	Normal	Normal
85	F	10-12 yr.	Canner	Sawdust (ext.)	Sawdust (ext.)
86	F	10-12 yr.	Canner	Telang (ext.)	Telang (ext.)
87	?	6 mo.	Prime	Telang	Telang (ext.) Sawdust
88	F	2 yr.	Prime	Normal	Sawdust
89	F	6 mo.	Prime	Telang Sawdust	Sawdust
90	?	2 yr.	Choice	Normal	Sawdust (sl.)
91	?	2 yr.	Prime	Normal	Normal
92	F	8-10 yr.	Good	Telang	Telang (mod.)
93	F	8-10 yr.	Canner	Telang	Telang (mod.)
94	F	4-6 yr.	Choice	Telang	Telang (ext.)

atrophy was noted in some of the hepatic cells adjacent to the distended sinusoids and many of the nuclei were pyknotic. The hepatic cords, in the same lobule, on the opposite side of the central vein were normal in appearance (fig. 3).

Histologic examination (fig. 5) of the macroscopic, purplish, pinpoint telangiectatic areas often revealed a later stage of the condition. This particular section was taken from a 6-month-old heifer exhibiting both "sawdust" and telangiectatic lesions. There was a leucocytic infiltration in addition to an increase in blood in the involved area. The hepatic cells in, and adjacent to, the lesion were undergoing cloudy swelling and hydropic degeneration. The histologic picture was one of an acute inflammatory reaction.

Another stage is represented by a section taken from a necrotic focus (fig. 6) which would be classified macroscopically as a "sawdust" lesion. This particular specimen was obtained from a 2-year-old heifer exhibiting moderate telangiectatic and "sawdust" lesions. A zone of hyperemia was noted about the necrotic focus which was undergoing leucocytic infiltration. Histologic examination of the average macroscopic "sawdust" lesion revealed a necrotic focus in which the hepatic cell outline was completely destroyed centrally (fig. 7).

Transitional stages between so-called "sawdust" and telangiectatic lesions often could be noted in the same specimen (fig 8). The necrotic focus (former "sawdust" lesion) was in a stage of resolution and repair. Necrotic foci, which were variable in extent and duration, were scattered diffusely throughout the section. The "sawdust" lesions were in various stages of degeneration, resolution, and regeneration (fig. 9). The periphery of many of the necrotic foci was penetrated by regenerating hepatic cords, illustrating the beginning of the formation of telangiectatic areas.

The classical telangiectatic picture was one of widely distended sinusoids giving a cavernous appearance (fig. 10). This section was taken from a 12- to 15-year-old grade Jersey Cow. The sinusoidal lumen may be increased ten to 50 times that of normal, yet the lining cells in most instances remain intact (fig. 11). Postmortem clotting of blood was conspicuous in the cavernous sinusoidal areas. However, the blood contained within the areas was normal in appearance. The hepatic cords adjacent to, and lining, the distended sinusoids appeared normal. No degeneration or necrosis was noted in the surrounding hepatic lobules although some of the sinusoids were dilated. The typical lobule structure was destroyed in the involved areas although the remaining hepatic parenchyma was normal in appearance; there was no increase in collagenous or elastic fibers in or about the telangiectatic lesions. Glisson's capsule appeared normal.

Frequently, an apparently normal liver exhibited, upon microscopic examination, the typical so-called "sawdust" and telangiectatic lesions. Necrotic foci (fig. 12) were noted microscopically; however, these areas could not be observed grossly. Of 17 livers classified as normal macroscopically, seven showed the beginning of "sawdust" and telangiectatic lesions microscopically.

Statistical Data.—The preceding tables include data on 82 liver specimens obtained from the examination by the writer of approximately 9,500 bovine livers. Table 1 lists the age, sex, condition of the carcass, and classification of the specimens. The ages were estimated by the teeth. In certain instances, the sex was not known because of circumstances beyond control, *e. g.* interruptions. Edelmann's[10] chart on the classification of food animals was used to classify the condition of the carcass. Under "Microscopic Classification" (table 1), descriptive terms are used as follows:

SAWDUST LESIONS

Slight (*sl.*).—Less than four or five lesions to an average 8-10-mm. square section.

Very slight (*v. sl.*).—Lesion usually single—small in size.

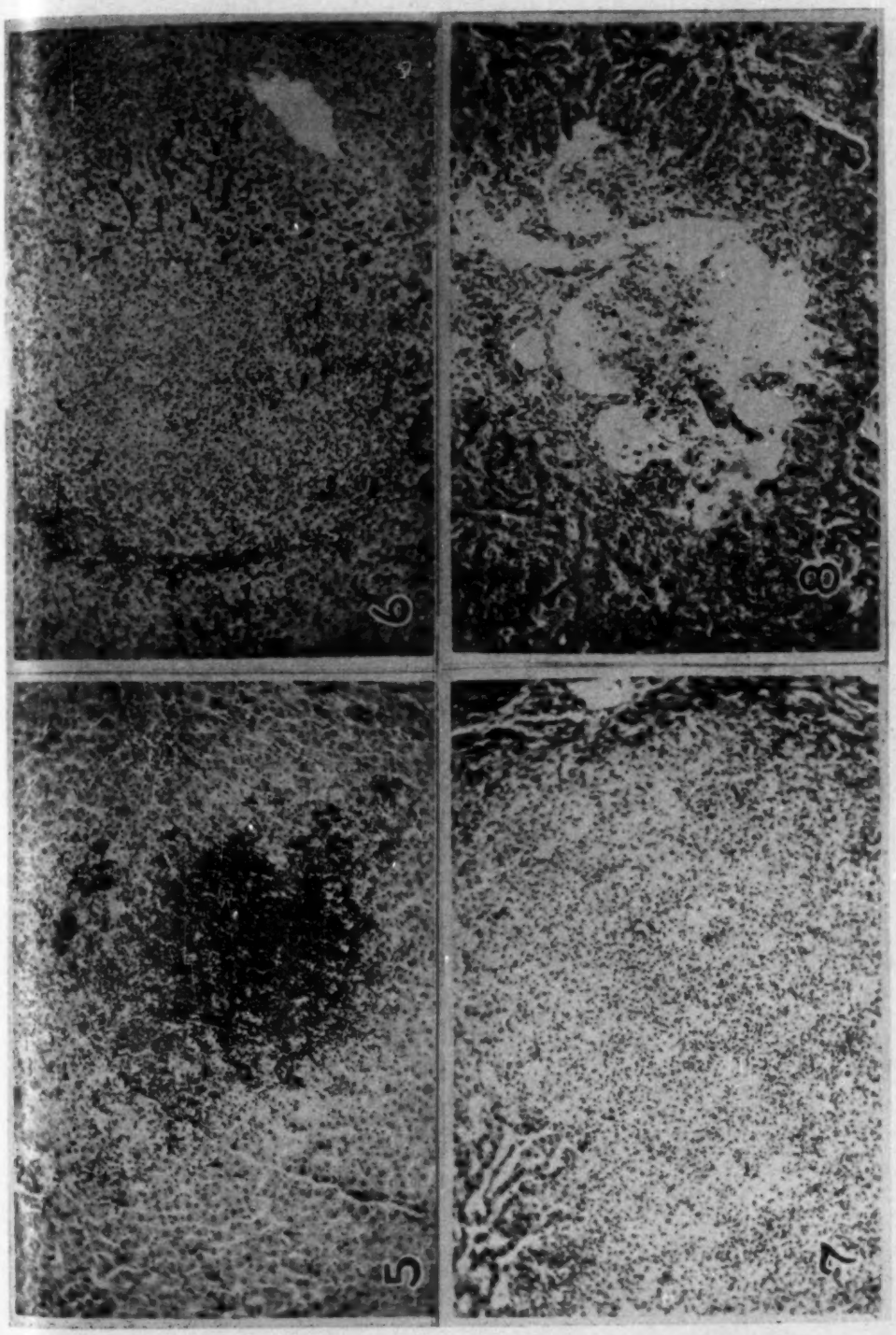


Fig. 5—The involved area is undergoing leucocytic infiltration. x 130. Specimen 89.
Fig. 6—Note the congested sinusoids surrounding the necrotic focus. x 130. Specimen 27.
Fig. 7—Microscopic picture of the average macroscopic "sawdust" lesion. x 130. Specimen 31.
Fig. 8—Transitional stage between the so-called "sawdust" and telangiectatic lesions. x 130. Specimen 31.

atrophy was noted in some of the hepatic cells adjacent to the distended sinusoids and many of the nuclei were pyknotic. The hepatic cords, in the same lobule, on the opposite side of the central vein were normal in appearance (fig. 3).

Histologic examination (fig. 5) of the macroscopic, purplish, pinpoint telangiectatic areas often revealed a later stage of the condition. This particular section was taken from a 6-month-old heifer exhibiting both "sawdust" and telangiectatic lesions. There was a leucocytic infiltration in addition to an increase in blood in the involved area. The hepatic cells in, and adjacent to, the lesion were undergoing cloudy swelling and hydropic degeneration. The histologic picture was one of an acute inflammatory reaction.

Another stage is represented by a section taken from a necrotic focus (fig. 6) which would be classified macroscopically as a "sawdust" lesion. This particular specimen was obtained from a 2-year-old heifer exhibiting moderate telangiectatic and "sawdust" lesions. A zone of hyperemia was noted about the necrotic focus which was undergoing leucocytic infiltration. Histologic examination of the average macroscopic "sawdust" lesion revealed a necrotic focus in which the hepatic cell outline was completely destroyed centrally (fig. 7).

Transitional stages between so-called "sawdust" and telangiectatic lesions often could be noted in the same specimen (fig 8). The necrotic focus (former "sawdust" lesion) was in a stage of resolution and repair. Necrotic foci, which were variable in extent and duration, were scattered diffusely throughout the section. The "sawdust" lesions were in various stages of degeneration, resolution, and regeneration (fig. 9). The periphery of many of the necrotic foci was penetrated by regenerating hepatic cords, illustrating the beginning of the formation of telangiectatic areas.

The classical telangiectatic picture was one of widely distended sinusoids giving a cavernous appearance (fig. 10). This section was taken from a 12- to 15-year-old grade Jersey Cow. The sinusoidal lumen may be increased ten to 50 times that of normal, yet the lining cells in most instances remain intact (fig. 11). Postmortem clotting of blood was conspicuous in the cavernous sinusoidal areas. However, the blood contained within the areas was normal in appearance. The hepatic cords adjacent to, and lining, the distended sinusoids appeared normal. No degeneration or necrosis was noted in the surrounding hepatic lobules although some of the sinusoids were dilated. The typical lobule structure was destroyed in the involved areas although the remaining hepatic parenchyma was normal in appearance; there was no increase in collagenous or elastic fibers in or about the telangiectatic lesions. Glisson's capsule appeared normal.

Frequently, an apparently normal liver exhibited, upon microscopic examination, the typical so-called "sawdust" and telangiectatic lesions. Necrotic foci (fig. 12) were noted microscopically; however, these areas could not be observed grossly. Of 17 livers classified as normal macroscopically, seven showed the beginning of "sawdust" and telangiectatic lesions microscopically.

Statistical Data.—The preceding tables include data on 82 liver specimens obtained from the examination by the writer of approximately 9,500 bovine livers. Table 1 lists the age, sex, condition of the carcass, and classification of the specimens. The ages were estimated by the teeth. In certain instances, the sex was not known because of circumstances beyond control, *e. g.* interruptions. Edelmann's[10] chart on the classification of food animals was used to classify the condition of the carcass. Under "Microscopic Classification" (table 1), descriptive terms are used as follows:

SAWDUST LESIONS

Slight (*sl.*).—Less than four or five lesions to an average 8-10-mm. square section.

Very slight (*v. sl.*).—Lesion usually single—small in size.

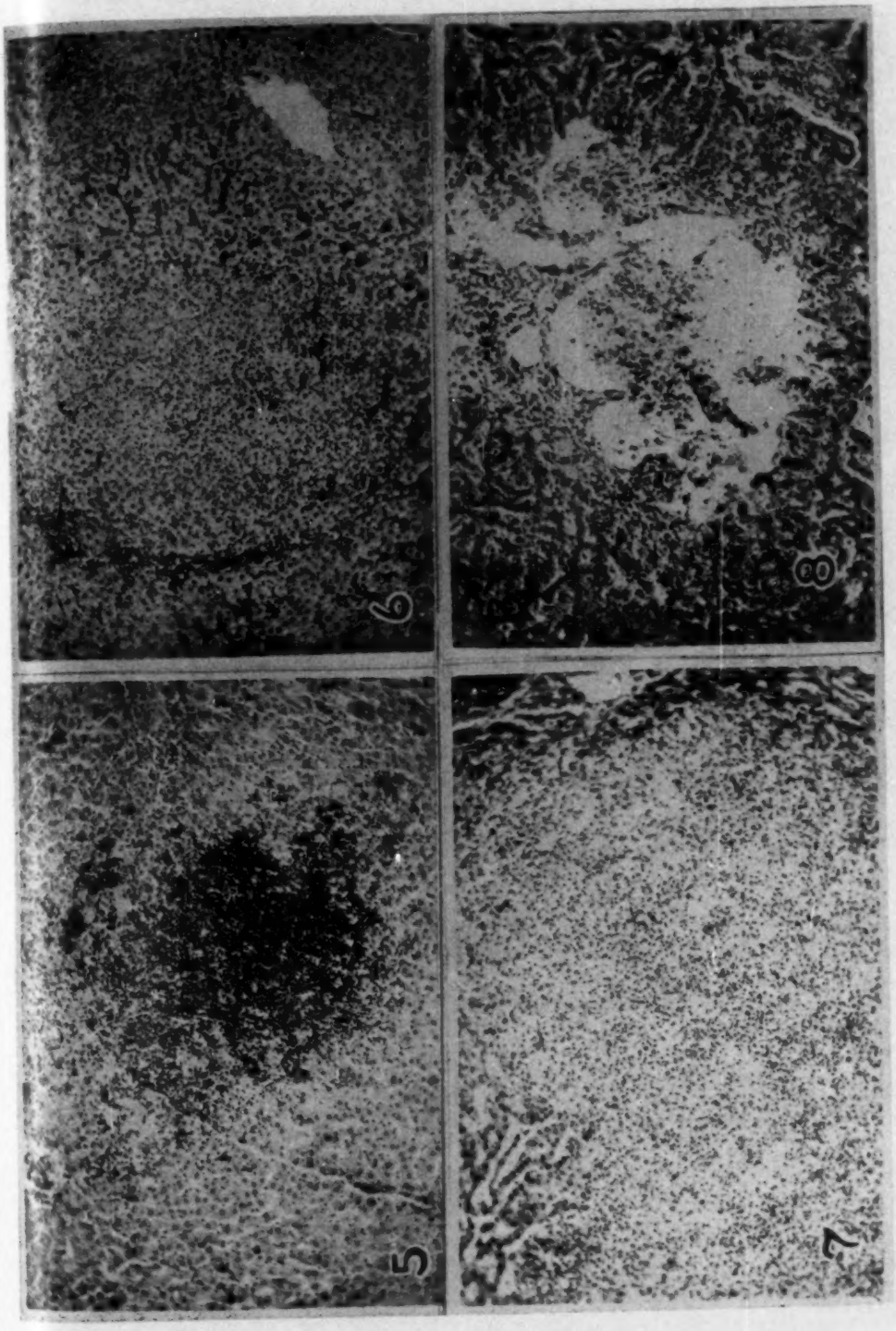


Fig. 5—The involved area is undergoing leucocytic infiltration. x 130. Specimen 89.
Fig. 6—Note the congested sinusoids surrounding the necrotic focus. x 130. Specimen 27.
Fig. 7—Microscopic picture of the average macroscopic "sawdust" lesion. x 130. Specimen 31.
Fig. 8—Transitional stage between the so-called "sawdust" and telangiectatic lesions. x 130. Specimen 31.

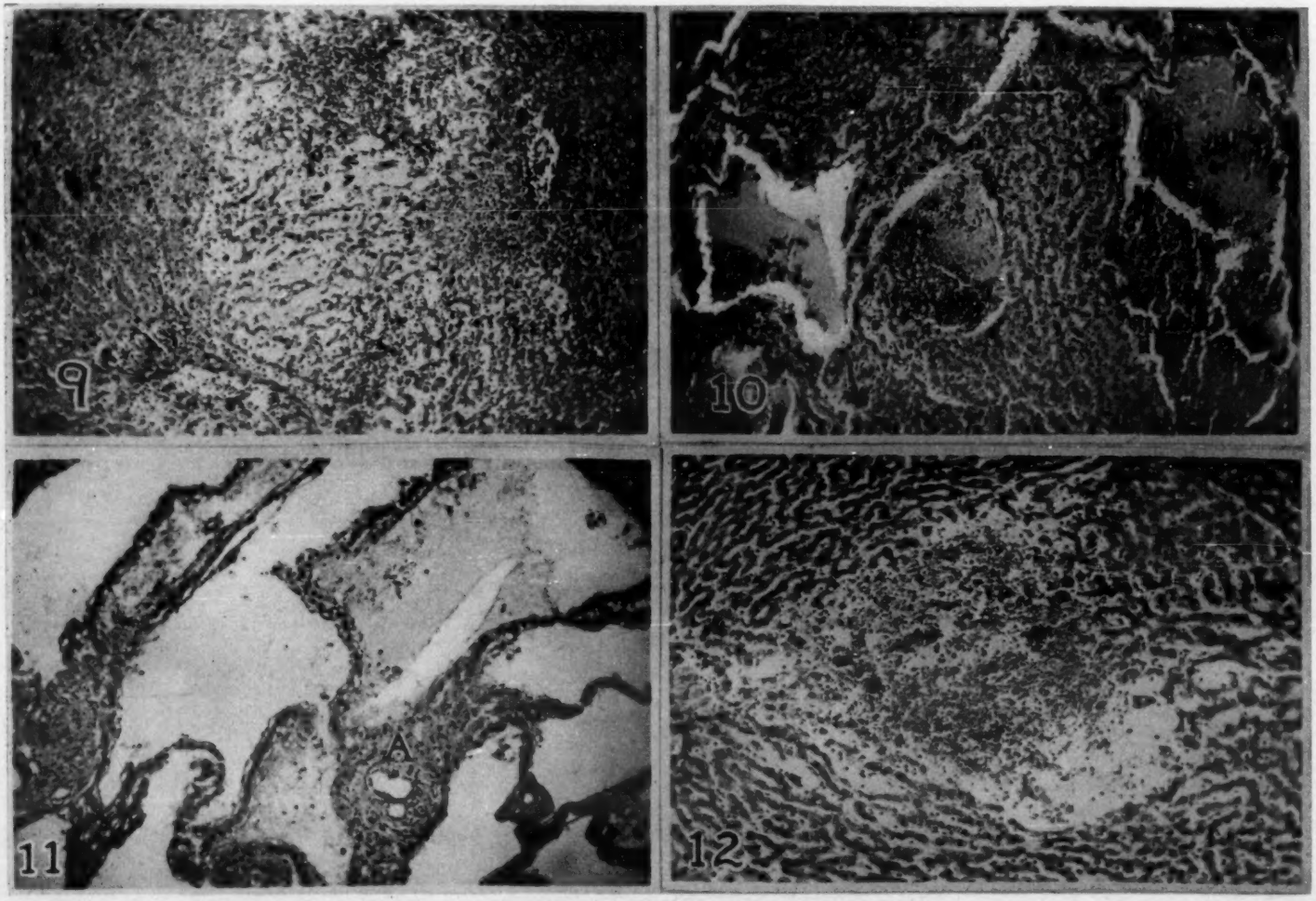


Fig. 9—Note the hepatic cords penetrating the necrotic focus and the apparent beginning formation of telangiectatic areas. x 100. Specimen 25.
Fig. 10—Note the greatly enlarged sinusoids which are typical of the classical telangiectatic lesion. The blood within the cavernous areas appears normal. x 130. Specimen 30.
Fig. 11—Note the intact lining cells of the dilated sinusoids of a classical telangiectatic lesion. The adjacent hepatic parenchyma appears normal. This particular lesion surrounds the portal canal area "A". x 130. Specimen 54.
Fig. 12—Photomicrograph of a "sawdust" lesion present in a liver classified grossly as normal. x 130. Specimen 41.

Moderate (*mod.*).—Lesions irregularly distributed involving not more than a third to a half total area of the section.

Extensive (*ext.*).—Lesions diffusely scattered; very little normal hepatic parenchyma remaining.

TELANG LESIONS

Slight (*sl.*).—Telang lesion usually single—small, involving an area not greater than five or six hepatic lobules.

Moderate (*mod.*).—One or more lesions, involving a fourth to a half of the entire section.

Extensive (*ext.*).—Lesion or lesions involving more than a half of the entire section.

DISCUSSION

The macroscopic examination of approximately 100 liver specimens exhibiting lesions and the microscopic examination of several thousand tissue sections gave the impression that the so-called "sawdust" and telangiectatic lesions represent different stages of a focal hepatitis and its termination.

The telangiectatic and "sawdust" lesions are variable in size and distribution. They may be confined to a single section of the liver or they may be distributed throughout the entire organ. The primary stage in the observed focal hepatitis is that of distended sinusoids engorged with blood (fig. 1). The involved areas are at first microscopic in size.

The small pinpoint telangiectatic foci may be easily overlooked. These purplish black lesions were most frequently noted in yearlings and 2-year-olds. They were also observed in several calves as listed in table 1. Areas of capillary congestion, resembling the first stage of inflammation, were noted microscopically (fig. 2). The second phase exhibited a leucocytic infiltration in addition to an increase in erythrocytes in the involved areas (fig. 5). In a still later stage (fig. 6), the hepatic cells of the involved area had undergone necrosis; the cell detail was lost within the central portion of the lesion. The sinusoids about the periphery of the lesions were distended and the necrotic foci revealed the cellular changes characteristic of acute inflammation. These necrotic areas represented a third stage in the condition and were classified macroscopically as "sawdust" lesions. They were variable in size and appeared to be centrally or peripherally located in a hepatic lobule; or two, three, four, or more adjacent lobules were involved, so that they completely destroyed the typical structural unit of the liver (fig. 7). A coalescing of the hepatic cells and erythrocytes, noted in some of the necrotic areas, gave a homogeneous, structureless appearance resembling hyaline degeneration.

The following stage, that of resolution and repair by regeneration, could frequently be noted in the same section. The necrotic foci which appeared macroscopically as "sawdust" lesions had undergone resolution. The sinusoids about the periphery of the lesions were distended, and the necrotic areas were penetrated by regenerating hepatic cords, endothelial lining cells, and a few fibroblasts. The microscopic picture gave the impression that a formation of telangiectatic areas was beginning at this stage (fig. 8, 9).

The classical telangiectatic lesion exhibited widely dilated sinusoids giving a cavernous appearance (fig. 10). This type of lesion was usually seen only in the older cattle. The hepatic cords adjacent to the dilated sinusoids appeared normal, although occasionally pressure atrophy of a secondary nature was noted. These findings

TABLE 2—Occurrence of Telangiectatic and Sawdust Livers in Relation to Condition of Carcass*

Condition of carcass	Microscopic classification T, S, or T+S†	Microscopic classification Normal
Fancy	2 livers	1 liver
Prime	31 livers	2 livers
Choice	15 livers	3 livers
Good	8 livers	1 liver
Utility	1 liver	0 livers
Canners	15 livers	3 livers

*Table includes specimens in which the age was unknown.

†T=Telangiectatic.
S=Sawdust.
T+S=Telangiectatic and Sawdust.

were in agreement with the reports of Stockman[5] and Schoti[17]. The lining cells and the blood within the cavernous areas appeared normal; no leucocytosis was observed in the chronic cavernous type of telangiectatic lesion. Jaeger[8] also concluded that the blood in the telangiectatic areas was normal. Contrary to the report of Chaussé[9], no excessive increase in connective tissue or elastic fibers could be noted in or about the telangiectatic areas with the exception, in a very few instances, of the large lesions seen in the livers of older cattle. The telangiectatic area was frequently periportal in location, and it can be seen how the interlobular connective tissue has, in the past, been incorrectly considered as a portion of the lesion (fig. 11). Glisson's capsule also appeared normal.

These microscopic observations suggest that apparently one condition exhibiting various stages is present. The small, purplish, pinpoint areas seen macroscopically are microscopically merely areas of distended sinusoids engorged with blood. This phase may be considered as the first stage of a focal hepatitis. The small, yellowish white ("sawdust") areas seen macroscopically are foci of necrosis. This intermediate stage exhibits a cellular reaction typical of acute inflammation. Since transitional stages exhibiting a picture of resolution and repair were frequently noted between the intermediate ("sawdust") stage and the classical telangiectatic areas, it is thought that the latter were the sequels of the local inflammatory reaction. This final telangiectatic picture was one of greatly distended sinusoids giving a cavernous appearance to the section. Macroscopically, the areas were much larger than those classified as pinpoint telangiectatic areas, the latter representing the first stage of an inflammatory reaction. Since one of the important characteristics of the liver is its capacity for restoration, it was not strange that the necrotic foci should be replaced by normal hepatic cords. When 75 per cent of the liver is removed from a dog, the remaining lobes rapidly increase by hyperplasia so that, in six to eight weeks, restoration is complete (Mann)[18].

The large number of calf livers condemned under federal supervision because of the so-called "sawdust" condition is noted in table 3.

The fact that the "sawdust" condition is more prevalent in calf livers is additional evidence to indicate that the "sawdust" lesion precedes the typical or classical telangiectatic lesion. It is interesting to speculate that, in all probability, the calf livers condemned as telangiectatic were of the pinpoint variety and would thus be classified microscopically as being in the first stages of an active hyperemia. This was found to be true in the livers examined in this study. Consequently, one would expect to find the large classical telangiectatic lesion in older animals, after they had

TABLE 3—Beef Livers Condemned under Federal Supervision from 1935-1944*

Fiscal Year	Animals slaughtered under Federal inspection†		Telangiectasis		Sawdust	
	Cattle	Calves	Cattle	Calves	Cattle	Calves
1935	9,853,000	5,719,000	212,021	632	80,070	4,180
1936	10,975,000	6,070,000	194,637	222	69,167	3,534
1937	10,070,000	6,281,000	193,218	255	55,561	2,977
1938	9,776,000	5,492,000	191,156	279	67,078	3,781
1939	9,446,000	5,264,000	194,874	458	70,147	3,679
1940	9,756,000	5,359,000	199,461	356	92,944	4,026
1941	10,946,000	5,461,000	210,573	393	91,813	5,453
1942	12,347,000	5,760,000	241,264	450	109,400	5,376
1943	11,727,000	5,209,000	222,801	333	107,283	4,859
1944	12,411,374	?	236,324	394	117,871	6,366

*Seher, O. W., U. S. Dept. Agric., Chief, Inspection Operations Section, Meat Inspection Division, Livestock Branch: Personal communication, November, 1945.

†Agricultural Statistics, 1944. U. S. Dept. Agric., U. S. Govt. Printing Office, Washington, D.C., 1944.

passed the pinpoint (first) stage and the "sawdust" (intermediate) stage. It is believed that the figures in table 3 aid in substantiating histopathologic findings.

In attempting to review briefly the possible etiologic factor or factors involved in the production of the condition, the following observations should be kept in mind. Neither the telangiectatic nor "sawdust" type of liver showed any particular breed distribution in the animals examined, nor were there any other concurrent lesions evident in the specimens studied. The liver and intestinal tract were examined for parasitism, but nothing of significance was noted. The carcasses, from which the "sawdust" and telangiectatic livers were obtained, were all passed as fit for human food. The systematic study of several hundred serial sections of beginning telangiectatic and "sawdust" lesions in 6-month-old calves revealed that the lesions were at first periportal (fig. 4). The portal canal area showed a typical inflammatory cellular reaction and the lesion at first seemed to be confined to a single hepatic lobule (fig. 3). Later, some of the lesion involved the entire hepatic lobule and four or five adjacent lobules; thus, the central vein was surrounded. Therefore, sections made at random through a lesion often proved misleading as no specific zonal arrangement could then be determined. However, the sections studied to date seem to indicate that the etiologic agent or factor gains entrance by way of the portal canal. In view of these findings, the possibility of an autointoxication by way of the portal venous system or biliary system should be kept in mind. Smith[19] presented data that rather conclusively suggest there is a definite relationship between ulcers of the rumen and abscesses of the liver in beef cattle.

Since the cytoplasm of the liver cell reflects, to some extent, the functional state of the cell (Maximow[20]), it is interesting to note that no constant correlation could be made between fatty deposition or glycogen deposition and the presence or absence of "sawdust" and telangiectatic lesions in the sections examined.

Table 1 reveals that, of the 58 animals in which both sex and age were known, the livers obtained were distributed as follows: 20 (34.4%) from heifers, 14 (24.1%) from steers, 21 (36.2%) from cows, and three (5.1%) from bulls. It should not necessarily be inferred from these percentages that the condition is more frequent in the female than in the male. It is quite possible that the sex of the animals slaughtered was unequal in the 9,500 carcasses examined. As one would anticipate, those carcasses classified as "good", "utility", and "canners" were from older cattle and, in most instances, they were females. As there are few old bulls slaughtered, there was lack of data on whether male animals in advanced ages are as susceptible as female animals. Table 1 reveals, however, that there is an equal distribution of the telangiectatic and "sawdust" livers between the sexes in the prime and choice grades. The distribution of "sawdust" and "telang" livers in relation to age was as follows: 6 mo. (4), yearlings (12), 18 to 24 mo. (25), 2 to 4 yr. (6), 4 to 8 yr. (3), 8 to 10 yr. (5), 10 to 12 yr. (6), 12 to 15 yr. (8), old bull (1), young bull (1). It was evident that the livers obtained from animals of all ages may exhibit "sawdust" and telangiectatic lesions.

It should be noted that, of the 17 livers classified as normal macrosopically in table 1, seven showed beginning "sawdust" and telangiectatic lesions upon microscopic examination (fig. 12). It seems quite probable that many livers now being passed for human food would exhibit microscopically the beginning lesions typical of a focal hepatitis.

An examination of table 2 reveals that the majority of the telangiectatic and "sawdust" livers were from carcasses classified as "prime" and "choice". This observation is in accord with the finding of Chaussé[3], Jaeger[8], Schoti[17], and others who reported that animals possessing telangiectatic livers were in good or very good condition. How-

ever, it should be noted in the same table that a considerable number of "canners" also exhibited the telangiectatic and "sawdust" lesions. The thought has frequently been advanced that the condition studied was confined mainly to young and well-fed animals. Since the lesions can be found in emaciated animals, "canners", and "cutters", one is led to believe that diet was not the sole etiological agent. Then too, "sawdust" and telangiectatic areas were observed in 6-month-old calves, which would tend to discredit the theory that the lesions may only result from metabolic changes due to nutritional disturbances incited by a "feeding-up" program. The heavy feeding of animals before slaughter could, however, increase the susceptibility of the hepatic parenchyma to injury from other causes by disturbing normal cell metabolism.

As was previously mentioned, the nutritional significance of beef liver has greatly increased with our scientific advances. Since Pavcek *et. al.*[21] have established the nutritional value of telangiectatic livers and reported the toxicity of certain "telang" livers when fed to weanling rats, the pathogenesis of this condition presents an ever increasing challenge.

SUMMARY AND CONCLUSIONS

1) Eighty-two beef livers exhibiting a focal hepatitis ("sawdust" and "telang" condition) were studied macroscopically and microscopically.

2) The distribution and the incidence of "sawdust" and "telang" livers in relation to sex, age, and condition of the carcass were determined.

3) The evidence to date indicates that apparently one condition, a focal hepatitis and its termination, is present. Therefore, it is thought that the so-called "sawdust" and "telang" lesions merely represent different stages of this condition.

4) Additional studies should be made in order to clarify and confirm the present classification of "sawdust" and "telang" beef livers, as given in this investigation.

5) The present views concerning the cause of "telang" and "sawdust" livers are a true reflection of the uncertainty of the meaning of the histologic picture.

6) Both "telang" and "sawdust" lesions can be noted macroscopically in the same liver and microscopically in the same section.

7) Transitional stages between "telang" and "sawdust" lesions are very evident in many of the sections.

8) Many livers, designated as normal grossly, exhibit "sawdust" and "telang" lesions microscopically.

9) No constant correlation could be made between fatty deposition or glycogen deposition and the presence or absence of "sawdust" and "telang" lesions in the sections examined.

10) No apparent increase in collagenous or elastic fibers was noted in or about the involved "telang" and "sawdust" lesions.

11) Serial sections studied to date seem to indicate that the lesions are at first periportal and that the etiologic agent or factor gains entrance by way of the portal canal.

12) Data recorded revealed that both the male and the female may be equally affected and that the livers obtained from animals of all ages may exhibit "sawdust" and "telang" lesions. Early stages of the condition were noted in 6-month-old calves.

13) The majority of the involved livers were from carcasses classified as "prime" and "choice". However, a considerable number of "canners" and "cutters" also exhibited the telangiectatic and "sawdust" lesions.

14) "Sawdust" and "telang" areas were observed in 6-month-old calves, emaciated animals, "canners", and "cutters", which would tend to discredit the theory that the lesions may only result from metabolic changes due to nutritional disturbances incited by heavy feeding of animals prior to slaughter.

15) All carcasses whose livers showed the lesions discussed were passed for human food; no concurrent lesions were evident.

16) There was no particular breed distribution in the animals examined.

17) It is apparent from this investigation that the gross classification of "sawdust" and "telang" livers is merely arbitrary.

18) This attempt to establish a definite histopathologic picture should facilitate further research and investigation as to the etiology, pathogenesis, and necessary preventive measures.

ACKNOWLEDGMENTS

The writer is grateful to Dr. E. A. Benbrook, of the Department of Veterinary Pathology, for his valuable counsel during the course of this investigation; to Dr. H. A. Smith, also of the Department of Veterinary Pathology, for the photomicrographs taken and helpful suggestions; and to Dr. H. L. Foust of the Department of Veterinary Anatomy, under whose direction this investigation was initiated.

In addition, the writer wishes to acknowledge his indebtedness to the following:

Dr. H. E. Kingman, Jr., of Wilson and Company, whose coöperation and helpful interest made it possible to obtain the specimens used in this investigation; all members of the Wilson organization whose complete coöperation contributed much; and various officials of the Meat Inspection Division of the Livestock and Meats Branch, Office of Marketing Services of the War Food Administration, United States Department of Agriculture, for their valuable assistance.

References

[1]National Live Stock Loss Prevention Board: Meat Wasted in Marketing Livestock. 1944 Report. 700 Livestock Exchange Bldg., Union Stock Yards, Chicago, Ill.

[2]Sisson, S., and Grossman, J. D.: The Anatomy of the Domestic Animals. Third edition. W. B. Saunders Co., Philadelphia, (1938): 472.

[3]McFadyean, J.: Cavernous Angioma of the Liver of the Ox. J. Comp. Path. and Therap., *3*, (1890): 345-346.

[4]Stubbe, L.: Des infarctus hemorrhagiques proprement dits du foie chez la bête bovine; anatomie pathologique et etiologie. Bull. Acad. Roy. de Med. de Belgique, series 4, (5), (1891): 659-667.

[5]Stockman, S.: Structure and Origin of Cavernous Angiomata in the Livers of Oxen. J. Comp. Path. and Therap. *9*, (1896): 320-322.

[6]Stroh: Ueber die fleckige Kapilläraektasie in der Leber der Wiederkäuer. Monatsh. f. prakt. Thierheilk. *14*, (1903): 133-186.

[7]Kitt, T.: Textbook of Comparative General Pathology. Authorized trans. by W. W. Cadbury, W. T. Keener Co., Chicago, (1906): 362-364.

[8]Jaeger, A.: Die Teleangiektasis der Leber der Bovinen. Arch. f. Wissensch. prakt. Tierheilk., *33*, (1907): 71-112.

[9]Chaussé, P.: Étude du foie tacheté hemmorrhagique des bovins. Rec. Med. Vet., *85*, (1908): 704-715.

[10]Edelmann, R., Mohler, J. R., and Eichhorn, A.: Meat Hygiene. Seventh edition. Lea and Febiger, Philadelphia, (1939): 229-231.

[11]Mallory, F. B.: Pathological Technique. W. B. Saunders Co., Philadelphia, (1938): 116-120.

[12]Salthouse, T. N.: Method for the Permanent Mounting of Sections Stained for Fat. Stain Technol., *18*, (1943): 143.

[13]Proescher, F.: Oil Red O Pyridin, a Rapid Fat Stain. Stain Technol., *2*, (1927): 60-61.

[14]Smith, J. L.: On the Simultaneous Staining of Neutral Fat and Fatty Acid by Oxazine Dyes. J. Path. and Bact., *12*, (1907-08): 1-4.

[15]Guyer, M. F.: Animal Micrology. Fourth edition, University of Chicago Press, (1936): 64-67.

[16]Bensley, R. R., and Bensley, S. H.: Handbook of Histological and Cytological Technique. University of Chicago Press, (1938): 52-53, 103-104.

[17]Schoti, F.: Beiträge zur Pathogenese der Teleangiektasie der Rinderleber. Inaug. Diss. Friedrich-Wilhelms Universität, Berlin, 1936.

[18]Mann, F. C.: The Cytology of the Liver and Its Functional Significance. In Cowdry, E. V.: Special Cytology. Second edition, vol. 1, Paul B. Hoeber, Inc., New York, (1932): 335-371.

[19]Smith, H. A.: Ulcerative Lesions of the Bovine Rumen and Their Possible Relation to Hepatic Abscesses. Am. J. Vet. Res., *5*, (1944): 235-242.

[20]Maximow, A. A., and Bloom, W.: A Text-Book of Histology. Third edition, W. B. Saunders Co., Philadelphia, 1938.

[21]Pavcek, P. L., Herbst, E. J., and Elvehjem, C. A.: The Nutritional Value of Telang Livers. J. Nutr., *30* (1), (1945): 1-9.

Average Levels of Various Constituents, Physical Properties, and Formed Elements of the Blood of Cows on Pasture

WERNER BRAUN, Ph.D

Berkeley, California

INFORMATION about average levels of blood constituents is of considerable value for diagnosis and experimental work, particularly in cattle where, until recently, knowledge has generally been based on relatively few data. The purpose of this paper is to put on record new information on average levels of 23 constituents, physical properties, and formed elements of the blood. These data were obtained during an experiment which had been designed primarily to test the effect of differences in vitamin A intake upon resistance to *Brucella abortus* infection. The experiment included a six-month period of blood analysis on non-infected animals maintained on natural grass pasture, and a subsequent five-month period during which the animals were maintained in four different dietary groups, including one pasture group[1]. During the latter period, the majority of the animals were exposed to *Br. abortus*. The data to be presented are based only on results obtained with animals on pasture, *i.e.*, all animals from March to September, and the small group of animals remaining on pasture after that date. These data permit an analysis of normal average blood levels on pasture and the effects of normal seasonal changes of the pasture, as well as the animals' age, upon some of these levels. The infection with *Br. abortus* had no effect on the blood constituents tested, except for decreases of the phosphatase level which have been reported elsewhere[2]. Data which were obtained around time of abortion or calving have not been included in this report in all cases where an effect on the respective blood constituents was observed during that period. All data here presented can, therefore, be regarded as normal for animals on pasture.

From the Department of Veterinary Science, University of California, Berkeley, Calif. This work has been supported in part by grants from the U.S. Bureau of Animal Industry and the Regents of the University of California.

MATERIALS AND METHODS

Animals.—Forty-nine cows were used in the experiment which lasted from March, 1942, to February, 1943. The ages of the animals varied from 18 months to 13 years. In the data to follow, animals which were less than 30 months of age on Sept. 1, 1942, are classified as "young animals" (24 animals), those which were older than 30 months on Sept. 1, 1942, are classified as "old animals" (25 animals). The majority were Holstein-Friesians; the rest consisted of 4 Jersey, 1 Shorthorn, 1 Hereford, and 7 crossbred animals.

Diet.—From March until Sept. 1, 1942, all 49 cows were maintained on nonirrigated, natural grass pasture in Strawberry Canyon, Berkeley, Calif., without supplementary feed. (For description of this pasture, *see* below). After September 1, the animals were divided into four groups with different dietary regimes. Eight of the cows remained on pasture. For data after September 1, results obtained from these 8 animals only are included in this report.

Description of the Pasture.—The Veterinary Science pasture in Strawberry Canyon is typical of the central California coast. The winter climate is mild and rainy, that of the summer months is rainless, but cool and foggy for the most part. August and September are often the warmest, driest months (*see* fig. 1). The pasture slopes steeply southward except at its west end, where it is on an equally steep west-facing slope, and along its eastern edge, where there is a gentle north slope. The soil is heavy clay, with a high water-holding capacity, but becomes hard, dry, and cracked in late summer.

The forage cover consists mainly of annual species introduced from Europe. The most important grass species are wild oats (*Avena*

TABLE I—Average Levels of 23 Constituents, Physical Properties, and Formed Elements of Cows' Blood

	Unit	All Ages			Young Animals			Old Animals		
		Average	Standard Deviation	Number of Samples	Average	Standard Deviation	Number of Samples	Average	Standard Deviation	Number of Samples
Hemoglobin	Gm./100cc. (B)	12.0±0.09	1.31	201	12.2±0.12	1.22	105	11.8±0.14	1.37	96
Glucose	mg./100cc. (B)	82.5±0.82	11.96	212	82.9±1.13	12.21	116	82.1±1.19	11.62	96
Total N	mg./100cc. (S)	1120±14.3	208.1	212	1070±19.1	205.7	116	1180±19.8	194.4	96
NPN	mg./100cc. (S)	34.0±0.53	7.36	191	34.6±0.74	7.35	100	33.4±0.77	7.30	91
Albumin	Gm./100cc. (S)	3.4±0.06	0.72	166	3.3±0.08	0.74	93	3.4±0.08	0.70	73
Globulin	Gm./100cc. (S)	3.7±0.09	1.19	172	3.5±0.12	1.16	91	3.9±0.13	1.19	81
Inorg. phosphate	mg./100cc. (S)	6.1±0.08	1.15	195	6.4±0.11	1.12	101	5.9±0.12	1.13	94
Serum phosphatase*	Bodansky units/100cc.	4.3±0.20	2.26	123	4.9±0.26	2.06	63	3.8±0.30	2.32	60
Inorg. sulfate	mg./100cc. (S)	3.4±0.14	1.76	153	3.1±0.17	1.57	84	3.7±0.23	1.92	69
Chloride [1] **	mEq./100cc. (S)	97.8±0.71	4.47	40	97.1±0.82	4.27	27	99.1±1.37	4.95	13
Chloride [2] **	mEq. /100cc. (S)	108.2±1.30	13.75	112	106.4±1.60	12.60	62	110.5±2.07	14.65	50
Vitamin C	mg./100cc. (P)	0.68±0.020	0.28	192	0.69±0.025	0.25	100	0.67±0.032	0.30	92
Carotenoids	mg./100cc. (P)	5.1±0.27	3.87	209	5.2±0.36	3.78	110	5.1±0.40	3.95	99
Vitamin A	I.U./100cc. (P)	50.1±2.34	32.21	190	49.5±3.19	31.62	98	50.8±3.42	32.78	92
Spec. gravity	gm./cc. (B)	1.0546±0.00039	0.00340	76	1.0553±0.00060	0.00371	38	1.0539±0.00047	0.00288	38
Viscosity	In multiple of H^2O (B)	5.87±0.068	0.846	154	5.80±0.097	0.885	83	5.95±0.094	0.789	71
Red cell percentage	% (B)	34.8±0.39	3.72	91	35.2±0.53	3.81	52	34.4±0.57	3.54	39
Erythrocytes	Millions/ cc. (B)	7.12±0.073	1.015	193	7.54±0.085	0.858	101	6.66±0.102	0.979	92
Leucocytes	per cc. (B)	9347±172.8	2374.9	189	9427±223.8	2193.2	96	9263±263.6	2541.7	93
Lymphocytes	% (B)	56.1±0.90	12.23	182	59.5±1.22	11.97	96	52.4±1.23	11.40	86
Monocytes	% (B)	7.8±0.48	6.41	182	6.9±0.68	6.65	96	8.9±0.65	5.95	86
Neutrophils	% (B)	27.0±0.80	10.72	182	25.4±1.09	10.68	96	28.6±1.15	10.63	86
Eosinophils	% (B)	8.4±0.49	6.95	182	7.5±0.70	6.86	96	9.3±0.74	6.87	86
Basophils	% (B)	0.7±0.08	1.14	182	0.7±0.11	1.12	96	0.8±0.12	1.15	86

(B) = whole blood; (P) = plasma; (S) = serum.
*, ** = [1]Based on data after Sept. 1 only; [2]based on all data; *based on data up to Oct. 2 only.

barbata and *Avena fatua*), Italian rye grass (*Lolium multiflorum*), soft chess (*Bromus mollis*), and foxtail barley (*Hordeum murinum*), with *Stipa pulchra* as the only perennial present in sufficient quantity to constitute an important part of the forage. The most valuable broad-leaved herbs are the annual alfilaria (*Erodium moschatum* and *Erodium cicutarium*) and bur clover (*Medicago hispida*).

Due to the annual nature of the forage plants and the seasonal cycle of rainfall (*see* fig. 1), abundant green feed is available only during the winter and spring months. The early fall rains and fogs in late September and October start active growth of the perennial *S. pulchra*, and of some sub-perennial stands of Lolium on the east side of the pasture, which has a slight slope facing north. This is much frequented by the cows in early fall, and provides them with a moderate amount of green feed until December when, as a rule, the annuals are large enough to provide more feed. The feed supply increases steadily from then on, reaching its peak in most seasons between the first and fifteenth of May. During June, the supply of green feed decreases rapidly, so that by the first of July there are available only the much reduced clumps of Stipa, and the dry remains of the annuals. Of the latter, the burs of Medicago are undoubtedly the most nutritious and rich in protein, with the seeds of *B. mollis* also present in sufficient quantities to provide some feed. During the late summer and early fall, animals used to being on pasture will also resort to feeding on dried leaves and fruiting heads of the spotted thistle (*Silybum marianum*). The lowest ebb in feed supply is approximately from July 15 to September 15.

This cycle of pasture feed is typical of the coast counties of central California, but the cool summers and the high water-holding capacity of the soil prolong the supply of feed later into the spring than is the case in non-irrigated pastures in the interior of the state. In addition, the early rains and mild winter temperatures (*see* fig. 1) result in a greater supply of feed in late fall and early winter than in most parts of California.*

Blood Tests.—Blood samples were obtained from each animals at least once every month from March, 1942, to February, 1943, and all tests were made on the same sample.

The carotenoid and vitamin A content of the plasma were determined according to Kimble[3]. Since a Cenco photometer was used in all determinations here reported, it was necessary to adjust Kimble's calculations by substituting 1.0 for 1.9 (1.0 = layer of solution through which light passes in Cenco photometer; 1.9 = layer of solution through which light passes in Evelyn photoelectric colori-

*Description of the pasture was prepared by Dr. L. Stebbins, Jr., of the Division of Genetics.

TABLE 2—Significance of Differences between Data Obtained until September 1, Based on an Analysis of Variance

	Differences between tests	Differences between age groups "young" vs. "old" animals	Differences between individual animals
Hemoglobin	not significant	significant[1]	highly significant[2]
Glucose	highly significant	not significant	not significant
Total N	highly significant	not significant	significant
NPN	highly significant	not significant	not significant
Inorg. phosphate	not significant	highly significant	not significant
Serum phosphatase	highly significant	highly significant	not significant
Chloride	not significant	not significant	not significant
Vitamin C	highly significant	not significant	highly significant
Carotenoids	highly significant	not significant	highly significant
Vitamin A	highly significant	not significant	significant
Leucocytes	highly significant	not significant	highly significant

[1]=5% level.
[2]=1% level.

meter used by Kimble). Vitamin C was determined according to Mindlin and Butler[4], except that a correction was applied for possible turbidity of samples by an additional reading of samples after complete reduction of the dye (through addition of a few drops of a concentrated ascorbic acid solution). The viscosity was determined with an Ostwald viscosimeter at 20 C.; specific gravity was determined by weight at 20 C.; red cell percentage volume was determined by hematocrit; all blood counts, including the differential classification of the leucocytes, were made according to Osgood[5].* All other determinations were made according to Hoffman[6]; for hemoglobin, the Sheard-Sanford method as described by Hoffman was chosen, for glucose, the ferricyanide method using Folin-Wu filtrate as described by Hoffman was used (for a discussion of glucose values obtained by this method and the "true" glucose concentration, *see* page 65 of Hoffman's book).

RESULTS AND DISCUSSION

1) *Average Normal Blood Levels on Pasture.*—Table 1 presents the average normal levels of blood constituents tested for animals on pasture. As pointed out in the introduction, these averages are based on data obtained from March to September, when all 49 animals were on pasture, and data obtained from 8 animals which remained on pasture after September 1. Data which were obtained around time of abortion or calving have not been included in all cases where an effect on the respective blood constituents was observed during that period. Also, data obtained for phosphatase levels after October 2, the date of exposure to *Br. abortus*, have not been used in the calculation of the average normal level because phosphatase levels were found to be significantly lower in infected animals[2]. In addition to the average levels, table 1 lists the standard error and the standard deviation of each average. The standard error, being a function of the standard deviation and the number of samples used, is given in order to indicate the reliability of each value. The standard deviation is listed in order to indicate the average nor-

*The blood counts were made by J. Makins and B. Fulfs, U. S. Bureau of Animal Industry agents.

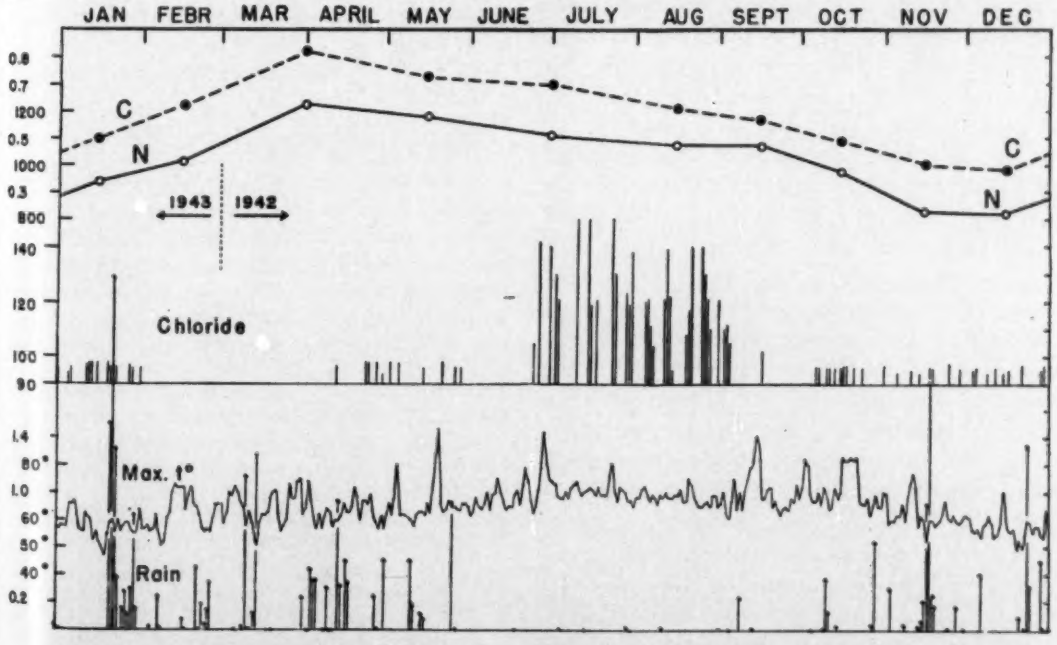


Fig. 1—A graphical representation of the following (top to bottom): (C) Changes in average blood levels of vitamin C (mg./100 cc.) in cows on pasture during a one-year period; (N) seasonal changes in average blood levels of total N (mg./100 cc.) in the same animals; (chloride) changes in the chloride level (mEq./100 cc.) of the blood (Note rise of chloride level during the dry period and return to constantly lower levels after the first rainfall of season); (Max. t°) daily maximum temperatures at Berkeley for one year; (rain) daily precipitation at Berkeley for one year.

is given in order to indicate the reliability of each value. The standard deviation is listed in order to indicate the average normal range of each blood level. The high standard deviations for total N, vitamins A and C, as well as carotenoids, are due to the seasonal changes of these blood levels, which are caused by the seasonal changes of the pasture. Such changes have been discussed in detail and have been illustrated for carotenoids and vitamin A in a previous report[1]. In figure 1 of the present report, these changes are illustrated for total N (mg./100 cc.) and vitamin C (mg./100 cc.).

Table 1 contains two columns for chloride (mEq./100 cc.), one based on data after September 1 only, the other for all data. The reason for this is the sudden temporary rise in chloride levels during June to September (*see* fig. 1). These high chloride levels occurred when the pasture became dry and, as figure 1 clearly illustrates, receded to constant levels of 98 mEq./100 cc. after the first heavy rainfall of the season. The inclusion of the high values obtained during these three months will, therefore, falsely elevate the average normal levels for chlorides throughout the year (column 2 of table 1), while, in reality the chloride level during the remaining nine months is constantly lower (column 1 of table 1).

2) *The Effect of Age of Animals and Changes in Pasture upon the Normal Blood Levels.*—In table 1, the average blood levels have been separately computed for young and old animals, and the effect of the animals' age on certain blood constituents can be noted. In addition, an analysis of variance[7] was made for many of the blood constituents, based on all individual data up to September 1*. Such an analysis permits the measurement of significance of that part of the variability whose origin is known. Thus, it was possible to test whether significant variations were produced in the blood levels by the animal's age, or by changes of feed available (seasonal variation) up to September 1, or by any significant (physiologic) differences between individual animals. (A similar analysis based on data after September 1 was not attempted, since too many variables existed after that date, such as different stages of pregnancy, infection, abortion, and parturition.) The results of the analysis, yielding the information sought regarding the significance of differences between individual animals, age groups, and tests (*i.e.*, seasonal variations), are summarized in table 2, which is self-explanatory.

*The advice of Dr. I. M. Lerner, Division of Poultry Husbandry, regarding the statistical treatment of the data, is gratefully acknowledged.

SUMMARY

Normal average levels of 23 constituents, physical properties, and formed elements of the blood for 49 cows on natural grass pasture are presented. The averages are based on data which have been collected during one year of periodic blood analyses, which included tests for hemoglobin, glucose, total N, NPN, albumin, globulin, inorganic phosphate, serum phosphatase, inorganic sulfate, chloride, vitamin C, carotenoids, vitamin A, specific gravity, viscosity, red cell percentage, as well as blood cell counts. The effect of the animal's age and seasonal changes of the pasture (diet) on some of these blood constituents are noted. Blood levels of total N, vitamins A and C, as well as carotenoids, showed pronounced seasonal changes. Chloride levels exhibited a sudden temporary rise during June to September, when the pasture became dry. The animal's age had a significant effect on blood levels of hemoglobin, inorganic phosphate, and serum phosphatase. A statistical analysis of differences between age groups, individual animals, and periodic tests (seasonal variation) is presented as further proof for such effects.

References

[1]Braun, W.: Studies on Carotenoid and Vitamin A Levels in Cattle. I. J. Nutr., *29*, (1945): 61-72.

[2]Braun, W.: Changes of the Serum Phosphatase Level after Infection of Cows with *Brucella abortus*. J. Immunol, *52*, (1946): 173.

[3]Kimble, M. S.: The Photocolorimetric Determination of Vitamin A and Carotene in Human Plasma. J. Lab. and Clin. Med., *24*, (1939): 1055.

[4]Mindlin, R. L., and Butler, M. A.: The Determination of Ascorbic Acid in Plasma; a Macromethod and Micromethod. J. Biol. Chem., *122*, (1938): 673-686.

[5]Osgood, E. E.: A Textbook of Laboratory Diagnosis. Third Edition, The Blakeston Company, Philadelphia, 1940.

[6]Hoffman, W. S.: Photelometric Clinical Chemistry. William Morrow & Co., 1941.

[7]Snedecor, G. W.: Statistical Methods. Iowa State College Press, 1940.

The Histopathology of Panleucopenia (Agranulocytosis) in the Domestic Cat

WAYNE H. RISER, D.V.M., M.S.

Des Moines, Iowa

MOST of the previous pathologic studies of panleucopenia (agranulocytosis) as described by Lawrence *et al.*[1] and Hammon and Enders[2] have dealt with the gross and microscopic appearances at the height of the disease or some time thereafter, but do not describe the development of the tissue lesions. It is the purpose of this paper, therefore, to show the succession of pathologic changes which occur in the stages during the course of the disease. The behavior of the peripheral blood elements and that of the intranuclear inclusions were described in previous papers[3, 4] and will not be discussed herein.

MATERIALS AND METHODS

Four healthy, well-nourished cats (females) were used to study the histopathology of panleucopenia (agranulocytosis). These cats were designated as 79, 80, 81, and 82. Tissues of these animals were compared with 2 normal animals designated as 77 and 78.

Cats 79, 80, and 81 were litter-mates, said to have been born on April 1, 1943. They were received at the laboratory on Oct. 8, 1943. All were females, all tabbies, and weighed 2,425, 2,210, and 2,445 Gm., respectively.

Cat 82 was also a tabby, female, and weighed 1,850 Gm. Its life history was not known. The source of the virus used was a spleen received on Oct. 14, 1943, from the Pitman-Moore Co. by air express, frozen in dry ice. Their cat, 3835, whose condition was diagnosed as panleucopenia, had been killed on Oct. 6, 1943. Inoculation was made the day the virus was received. The spleen, ground with sterile sand and Locke's solution to make a 20 per cent suspension, was centrifuged for thirty seconds at about 2,000 r.p.m. Five-tenths cubic centimeter of the supernatant fluid was injected intraperitoneally. After each injection, a drop or two of the fluid was cultured on nutrient agar. All cultures remained negative for four days, but the culture from cat 80, on the fifth day, showed two small colonies. One was identified as *Staphylococcus albus*, and, although the second could not be identified by the tests, it was not considered to be a common pathogen. Cats 77 and 78 were young, healthy cats obtained from a farm at which there was no history of previous infection. They were killed and tissues placed in preservative immediately upon arrival at the laboratory.

It was planned to sacrifice the experimental cats at intervals of three days. At each period, the animal which seemed the sickest was chosen on the basis of leucocyte count and general reactions.

Most of the tissues selected were fixed in Zenker's fluid with 5 per cent acetic acid. For some of the earlier clinical cases, 10 per cent formalin was used. The stains employed were hematoxylin and eosin, Giemsa's, phloxine and methylene blue, Masson's trichrome stain, and Feulgen's thymonucleic acid reaction, with or without a counterstain of fast green or orange G. As Giemsa's stain was variable in its results, it seemed to be influenced by the time the tissues had remained in Zenker's fluid. The phloxine and methylene blue stain was so active that polychromatic effects were obliterated. Delafield's, Harris', or Ehrlich's acid hematoxylin were all satisfactory. The commonly used eosin *Y* was better than ethyl eosin, in that the latter, like phloxine, was too active in its staining affinities. Feulgen's technique was used to determine whether thymonucleic acid was present in the inclusion bodies and thereby obtained an evaluation of the tinctorial reactions of eosin and Giemsa's stains. Clinical data for the weights, temperatures, and blood counts on cats 79 to 82 are found in table 1.

LYMPH NODES AND SPLEEN

Considering the small numbers of animals used, it was impossible to establish a criterion for the identification of the individual cells involved in the sequences that favor the vast proliferation of tissue in the lymph nodes and spleen.

This article is composed from a portion of: Riser, W. H.: The Diagnosis of Panleucopenia (Agranulocytosis) in the Domestic Cat. Master's Thesis, Iowa State College, 1945.

I am indebted to Dr. W. S. Gochenour of the Pitman-Moore Co. for preparing this material; to Dr. R. A. Packer of the Department of Veterinary Hygiene, Iowa State College, who ran the identification tests.

TABLE I—Clinical Data on Cats 79 to 82

	Weight in Gm.				Rectal temperature				Total white blood cells per cmm.			
	Cat no.				Cat no.				Cat no.			
Date	80	82	81	79	80	82	81	79	80	82	81	79
Oct. 11	2210	1850	2445	2425	102.8	102.4	102.2	103.0	9,700	16,175	17,625	11,250
12	2355	1800	2170	2280	102.4	102.2	103.0	102.8	18,800	14,900	13,600	14,575
13	2330	1810	2175	2355	103.2	102.3	102.5	102.4	21,850	16,000	12,000	13,350
14	2090	1720	2285	2240	103.6	102.6	103.4	103.0	9,800	16,100	12,550	8,800
15	2440	1785	2290	2465	102.4	101.3	102.4	101.3	22,100	12,250	15,200	16,950
16	2245	1745	2345	2360	105.0	104.0	104.4	101.4	5,500	9,250	9,550	15,400
17	2285	1720	2135	2260	104.0	103.0	103.0	102.9	4,550	6,150	5,800	12,200
18		1680	2095	2245		102.4	102.0	102.2		4,550	7,600	3,850
19		1750	2065	2320		102.8	103.4	103.2		5,800	6,000	3,950
20		1700	2025	2225		102.3	101.8	100.0		2,300	6,100	4,000
21			1925	2160			103.0	102.5			3,150	2,250
22			1780	2020			106.3	105.3			200	350
23				1965				103.2				1,450
24				1905				101.0				17,200
25				1830				102.0				25,400
26				1780				102.0				13,100

Differential count of leucocytes

	Cat 80*				Cat 82†				Cat 81*				Cat 79*			
Date	Neut.	Lym.	Mon.	Eos.	Neut.	Lym.	Mon.	Eos.	Neut.	Lym.	Mon.	Eos.	Neut.	Lym.	Mon.	Eos.
Oct. 11	45	52	2	1	44	42	3	11	43	52	1	4	52	42	3	3
12	44	55	1	0	40	45	2	13	44	48	1	7	53	46	1	0
13	33	67	0	0	48	37	2	13	42	53	3	2	49	45	2	4
14	39	56	1	4	75	21	1	3	34	62	1	3	38	62	0	2
15	40	52	3	5	48	38	1	13	26	72	1	1	44	50	4	2
16	56	37	1	6	59	29	2	14	86	20	4	8	60	36	0	4
17	68	25	0	7	70	22	0	8	36	60	2	2	88	9	1	2
18					48	45	1	6	48	49	0	3	41	49	8	2
19					69	20	0	11	53	43	4	0	44	54	0	2
20					21	73	0	6	47	50	1	2 ‡	22	75	1	2
21									6	90	2	2 ‡	24	70	6	0‖
22									0	95	0	5§	4	48	8	40¶
23													24	48	12	16#
24													84**	15	0	1
25													89**	10	0	1
26													82**	15	2	1

Inoculation of virus was made after the records were obtained on October 14.

*At necropsy there were found 1 ascarid and 1 tapeworm in cat 79, 4 ascarids and 1 tapeworm in cat 80, and 1 ascarid in cat 81.

†Cat 82 revealed coccidial oöcysts at fecal examination.

Percentages were based on less than 100 cells: ‡50 cells; §20 cells; ‖27 cells; ¶25 cells; #50 cells.

**All granulocytes were juvenile forms.

The hyperplastic tissue developed rapidly in the spleen and mesenteric lymph nodes

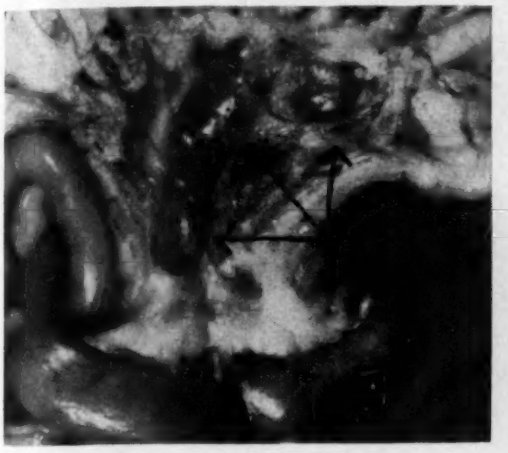


Fig. 1—Enlarged, hemorrhagic, edematous mesenteric lymph node found opposite the terminal ileum.

from the connective tissue and reticular cells in these organs.

Lawrence *et al.*[1, 5] and Hammon and Enders[2], in their descriptions, called this tissue "reticulo-endothelial tissue" and the hypertrophied cells "tissue monocytes". In

Fig. 2—Lymph node (mesenteric) from healthy cat. Note small number of secondary nodules. x 42.

this investigation, the same terms will be used. The pathologic changes in these tissues will be described as seen in cat 80, inoculated three days; cat 82, inoculated six days; cat 81, inoculated eight days; and cat 79, inoculated twelve days. The histopathology of these animals will be compared with cat 77 and cat 78 as normals.

In panleucopenia, the changes which

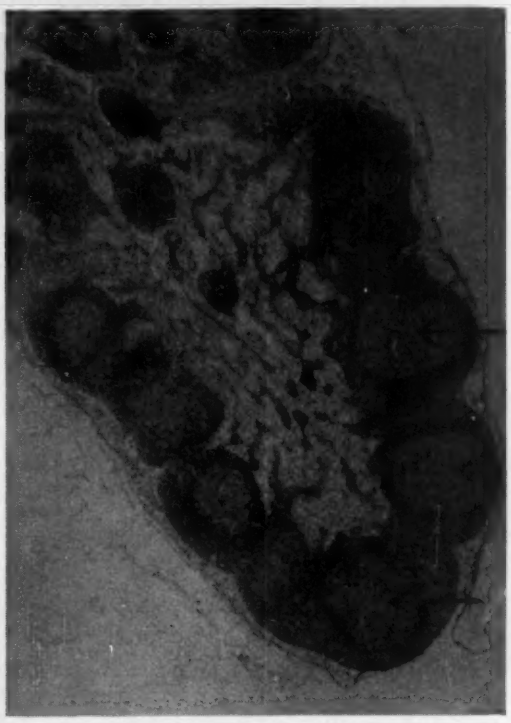


Fig. 3—Lymph node (mesenteric) from cat with panleucopenia (agranulocytosis) of three days' duration. Note extensive increase in number and cellular hyperplasia of secondary nodules. x 42.

took place in the tissues of these two organs were very rapid. In three days after inoculation, even before the animal showed clinical signs of illness, the mesenteric lymph nodes were grossly swollen, edematous, hyperemic (fig. 1), and, microscopically, the hyperplastic cells within the node markedly changed the pattern.

The secondary nodules at three days (cat 80) appeared in great numbers in the cortex (fig. 3). The tissue of the secondary nodules of the affected node appeared to be made up of lymphocytes, reticulo-endothe-

lium, and tissue monocytes. The reticulo-endothelium stained reddish pink with hematoxylin-eosin. Monocytes were very numerous, averaging 12.7 per field, and 4.87 per cent of these contained inclusions (table 2).

Fig. 4—Lymph node (mesenteric) from cat with panleucopenia (agranulocytosis) of six days' duration. Note extensive increase in number of hyperplastic secondary nodules with wide bands of small lymphocytes around the periphery. x 42.

It appeared that the lymphocytes were being crowded to the periphery of the follicle, forming a wide lymphocytic band

TABLE 2—Histopathology of the Mesenteric Lymph Nodes

Cat no.	Average monocytes per field*
Cat 77 Normal	3.0
Cat 78 Normal	2.9
Cat 80 3 days	12.7
Cat 82 6 days	10.7
Cat 81 8 days	2.5
Cat 79 12 days	1.0

*Average monocyte count was from 15 fields in the cortical region without regard to the secondary follicles.

three or four times wider than was seen in the normal node (fig. 2). No significant change was seen in the rest of the node except for a limited amount of hyperemia and a slight amount of reticulo-endothelium scattered throughout the lymphoid tissue. Many phagocytic cells were seen engulfing erythrocytes in the medulla.

In cat 82 (6 days) the lymphocytic bands about the secondary nodules were about twice the thickness of the three-day node (fig. 4). The reticulo-endothelium in the nodules stained dark bluish red with hematoxylin-eosin. Practically all the lymphocytes had disappeared from the interior of the follicle. Inclusions were formed in 6 per

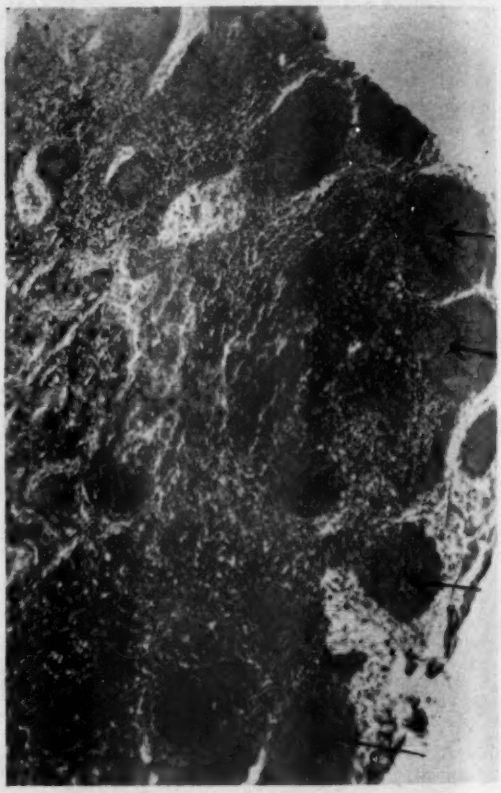


Fig. 5—Lymph node (mesenteric) from cat with panleucopenia (agranulocytosis) of eight days' duration. Note the absence of the lymphatic bands around the secondary nodules, and the increase of reticulo-endothelial tissue throughout the pulp. The secondary nodules show early central necrosis. x 42.

cent of the monocytes, but the number of monocytes had decreased slightly to 10.7 per field (table 2). Remnants of dead cells were seen in the darker staining areas.

The hyperemia had increased in the medullary region and large numbers of erythrophagocytic cells were found throughout the node, especially in the area between the capsule and the cortex (fig. 6). A small amount of hemosiderin was scattered throughout the tissue. There was reticulo-endothelial infiltration in the lymphoid tissue outside the secondary nodular areas.

The lymph node of cat 81 (8 days) was hyperemic and mildly hemorrhagic. Hemosiderin increase was noted in the medullary portion, but the erythrophagocytic cells had

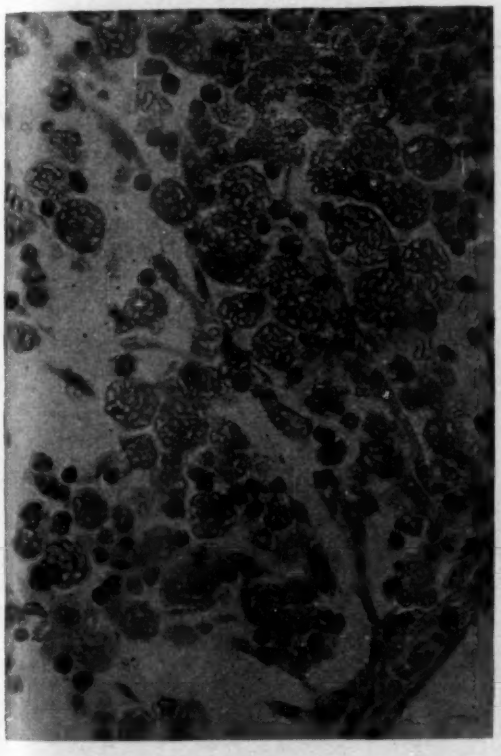


Fig. 6—Erythrophagocytosis in mesenteric lymph node of cat with panleucopenia (agranulocytosis) six days after inoculation. x 480.

disappeared. The lymphocytic band of the secondary nodules had been lost, and the follicular reticulo-endothelium stained a muddy, dark red (hematoxylin-eosin). There was necrosis of some of these cells (fig. 5). Only a few monocytes were left in the nodules, but occasionally a nucleus contained an old inclusion.

While the reticulo-endothelium was disappearing from the follicles, it had increased elsewhere in the nodes until it equaled the lymphoid tissue in volume. Inclusions were found in 3 per cent of the monocytes, but the height of the hyperplasia development had passed, for, monocytes were not as numerous as earlier in the disease. An average of only 2.5 monocytes was found per field compared to 12.7 at three days and 10.7 at six days.

Cat 79 (12 days) was recovering from the disease when the tissues were taken. The follicles were fast losing the reticulo-endothelial tissue and it was being replaced by lymphocytes (fig. 7). The lymph cells first appeared at the center of the follicle and spread toward the periphery as they increased in number. The sequence of events during regeneration or repair was the reverse of that seen during hyperplasia.

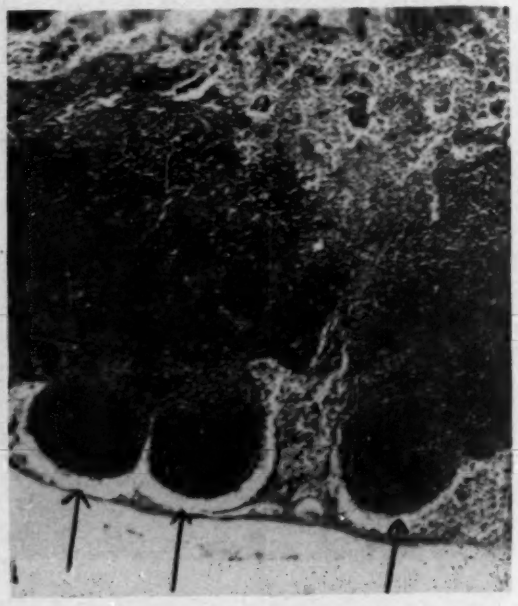


Fig. 7—Lymph node (mesenteric) from cat with panleucopenia (agranulocytosis) of twelve days' duration. Note the increase of lymphoid cells replacing the necrosed reticulo-endothelial cells in the center of the secondary nodules as shown in figure 5. x 42.

The hyperplastic tissue scattered through the nodes stained dark, muddy red (hematoxylin-eosin) as in the follicular tissue at eight days. The monocytes were disap-

pearing, for only 1 per field was found, and inclusions, ghost cells, and plasmasome nuclei were entirely absent.

Two lymph-node sections taken from a dying clinical case showed the secondary nodules almost repaired and free from reticulo-endothelium, but much of this tissue still remained infiltrated into the lymphoid tissue in other areas of the nodes (fig. 8 and 9).

Fig. 8—Lymph node taken from cat dying from panleucopenia (agranulocytosis). Note normal appearing secondary nodules with extensive invasion by hyperplastic reticulo-endothelial cells and the hyperemia throughout the node. x 42.

The histopathology of the spleen was very similar to that of the lymph nodes, although the secondary nodular areas were not so prominent and numerous as in the latter. The reticulo-endothelial tissue was evenly distributed through the pulp. The hyperemia and edema were similar to those of the lymph nodes. Many hypertrophied monocytes and inclusions were seen. Many neutrophils and phagocytes invaded the tissue.

INTESTINAL TRACT

The gross and microscopic damage which occurred in the gastrointestinal tract varied from no damage in mild cases to complete desquamation in severe ones of long duration. When inflammation and degeneration were noted they were greatest in the last 4 to 6 inches of the ileum.

Pathologic changes in the intestinal tract did not appear as early as in the adjoining lymph nodes and spleen, for, in the latter at three days, there was extreme reticulo-endothelial hyperplasia in the secondary nodules with numerous inclusions in the monocyte nuclei; whereas, in the ileum at three days (fig. 12), changes were limited to a striking mucous-cell increase in the epithelium and in the appearance of a small amount of mucin around the tips of

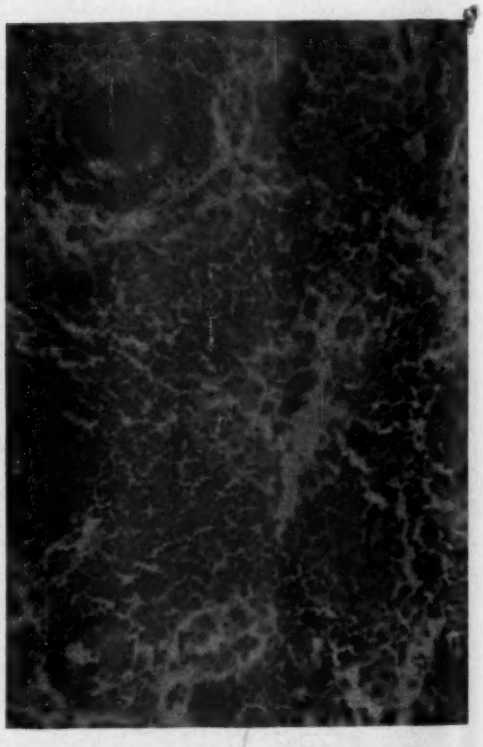


Fig. 9—Higher magnification of lymph node cortex of figure 8 taken outside of the secondary nodule. Note proliferation of reticulo-endothelial tissue and hyperemia. x 250.

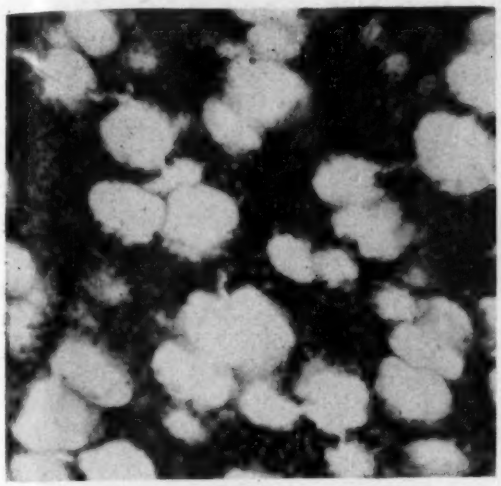


Fig. 10—Decalcified bone marrow section of proximal end of femur from a healthy cat with peripheral blood count of 7,250,000 erythrocytes and 16,000 leucocytes per cubic millimeter. x 250.

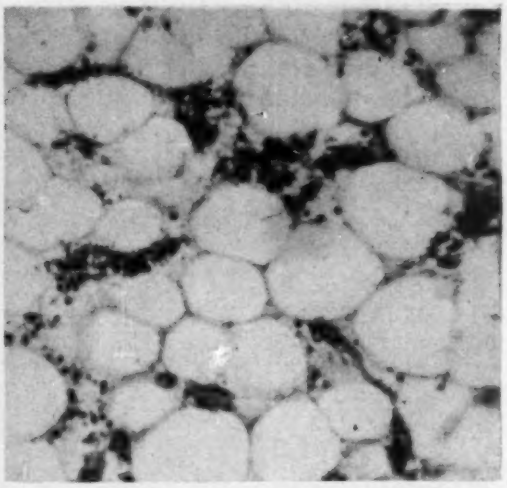


Fig. 11—Decalcified bone marrow section of proximal end of femur from a cat with panleucopenia (agranulocytosis) with a peripheral leucocyte count of 350 cells per cubic millimeter. Note the hypoplastic state of the cellular reticulum. x 250.

Fig. 12—Ileum of cat 80 at three days showing mucous-cell increase, also a small amount of mucin around the tips of the villi, some of which show beginning death in the lamina propria. x 100.

Fig. 13—Ileum of cat 82 at six days showing marked decrease in mucous production and necrosis extending deeper into the villi with serous exudate forming in the lamina of the bowel. x 100.

the villi. Here the cell nuclei were somewhat shrunken, but there was little change in the chromatin picture. Mild, cloudy swelling appeared in both the epithelial and

Fig. 14—Ileum of cat 81 at eight days showing disappearance of mucous cells except toward the lumen. Death changes including pyknotic nuclei are in evidence in most of the surface epithelium. The villi are becoming shorter, and rounding up. There is an attempt at repair as indicated by regenerating epithelium. x 100.

lamina propria cells in this area. The intestinal epithelial nuclei showed little or no hypertrophy.

In the ileum at six days (fig. 13), there was a marked decrease in mucus production in the region of the crypts, but out farther on the villi, many mucous cells were still functional. The mucinous exudate around the villi had changed to a serous exudate and had increased in amount until it almost filled the lumen of the bowel. Practically all of the nuclei of the epithelium, except those out on the tips, were hypertrophied. Many intranuclear inclusions were present on the lateral and basal portions of the crypts. Necrosis extended deeper into the villi, and the dead cells fell away from the live structure into the exudate.

At eight days (fig. 14), practically all mucous cells had disappeared except an occasional one out toward the lumen. The glandular cell cytoplasm had rather a cloudy, granular acidophilic appearance when stained with hematoxylin-eosin. The clear cut differentiation between the basophilic and acidophilic staining properties seemed to be gone. Changes which occurred in the cells after death and pyknotic

Fig. 15—Ileum of cat 79 killed during recovery, twelve days after infection. The surface epithelium shows beginning regeneration. Note increase of mucus production, also mitosis of glandular epithelium. x 100.

nuclei were in evidence in each acinus. The living nuclei showed all degrees of hypertrophy and the even, columnar arrangement of the cells was rapidly lost. The villi had rounded up in an attempt at repair as the fast-dying cells fell away. The ser-

ous exudate in the lumen became almost pseudomembranous in character and it adhered to the rounded villi. The exudate contained numerous dead cells. Many mitotic figures occurred in the epithelium of the acini and many of the nuclei were laden

Fig. 16—Ileum of cat 26 showing almost complete degeneration of mucosa; only short stubby and conical villi remain. x 100.

with inclusion bodies. Mitotic figures were not seen out on the tips, so the count was not raised. Large numbers of neutrophils and other phagocytes were found invading the acini and stroma. Hyperemia and edema were marked thoroughout the tissue.

Death of the villi in this disease was the result of coagulation necrosis. For this reason, there was no bleeding from the denuded villi, as seen in diseases in which the epithelium is digested away by liquefaction necrosis.

The degeneration continued and, at twelve days in some areas, only short, stubby, and conical villi remained (fig. 15). Some had almost disappeared. The nuclei of the intestinal epithelium were closely approximated and shrunken. The nuclear hypertrophy had disappeared and basichromatic flecks were scattered throughout the nuclear lymph. This was a return toward a normal nuclear configuration. Many epithelial cells, both of the glands and villi, were changed to mucous cells. Mitotic figures were numerous throughout. The cytoplasm was still granular, but it was not nearly so cloudy as it was at eight days. Infiltration of neutrophils and phagocytes was still great. The pathologic picture gradually shifted from one of death to one of beginning repair. Cat 79 was on its way to recovery when destroyed (*see* clinical chart), but if the disease had continued to be overwhelming, the epithelium of the ileum probably would have gone to complete desquamation as seen in figures 16 and 17, which were taken from 2 cats dying with the disease.

BONE MARROW

Bone-marrow smears from normal and diseased live animals and decalcified sections obtained at necropsy were used for this study. It was necessary to compare two slides from cats not studied in the experiment in order to describe the changes in the bone-marrow activity prior to the aplastic phase, because all the experimental animals outlived this period. Sections and blood smears were studied daily during the entire course of the infection.

The normal, average, differential marrow count as suggested by Lawrence *et al.*[5] was used as a basis for comparison.

No marked change in the differential marrow counts was found until the leucocytes of the peripheral blood dropped below 5,000 cells per cubic millimeter. This occurred between the fourth and fifth days following inoculation. The smears showed very few stab or segmented neutrophils at this time, but many young undifferentiated myeloid cells were seen.

But few cells other than erythrocytes were found in the bone-marrow smears at the aplastic stage. The exception was an occasional eosinophil, lymphocyte, or very young unclassified cell.

Young myeloid cells were plentiful in the smears on the first day of recovery. These were embryonic, and only an occasional segmented neutrophil was found. The proportion of mature cells increased daily and,

by the fourth day, the count was again normal.

When the decalcified sections were examined, it was found that those taken at the height of the disease showed the bone marrow to be aplastic and nonfunctional (fig. 11). Clear, rounded areas resembling fat occupied at least 70 per cent of the section. The apparent increase in the fat was the result of rapid atrophy of myeloid tissue by failure of maturation of the embryonic blood elements. Most of the cells seen in the reticulum were erythroid in nature with an occasional megakaryocyte and a few undistinguishable cells.

Sections taken the first day after recovery showed a large increase in the number of cells in the reticulum. The spaces were widened as a result of proliferation of the embryonic elements and, by the fourth day, the marrow appeared normal (fig. 10).

Fig. 17—Ileum showing complete desquamation of epithelium and almost complete loss of mucosa. x 100.

OTHER ORGANS

With the exception of the occasional presence of hyperemia, no significant changes were found in the lungs, urinary bladder, adrenal gland, salivary glands, or heart. Sections from liver, pancreas, and kidney for the most part were normal but, in those cats which experienced a long, stormy course of the disease, considerable tissue destruction was seen. Tissue taken from other cases, the histories of which are not described in this paper, showed marked parenchymatous destruction. This was particularly true of 1 case which had recovered from the virus phase when death occurred. The leucocyte count was at a level of 16,600 cells per cubic millimeter. In the liver sections, the parenchymatous cells were greatly damaged. All degrees of cloudy swelling, fatty degeneration, and necrosis could be found, and fully 60 per cent of the cells were destroyed. The kidney sections showed almost the same degree of destruction.

DISCUSSION

In the normal lymph nodes (fig. 2), only an occasional secondary nodule was present, and the remaining cortical area was uniformly studded with lymphoid cells. Drinker and Yoffey[6] were of the opinion that the number of secondary nodules and even the total number of cells in a lymph node depend upon the stimulus of an in-

fection. Where the stimulus so demanded, the cells and the secondary nodules reacted in response to the infection.

Drinker and Yoffey[6] also stated that Glimstedt (1936) raised 8 bacteria-free guinea pigs and that the lymphoid tissue found in these animals was only about 25 per cent of normal. He concluded, therefore, that infections were the normal stimulus to the growth of lymphoid tissues. He was also of the opinion that the germ centers were "reaction centers," since they did not develop in the absence of infection.

Taliaferro and Mulligan[7] stated that the connective tissue and reticular cells retained more or less the characteristics of embryonic mesenchyma but, upon proper stimulation, gave rise to a variety of cells, the type of which depended upon the type of stimulation. Maximow and Bloom[8] believe these cells can give rise to lymphocytes, granulocytes, erythrocytes, the fibroblasts of inflammation, and many other kinds of fixed tissue cells if stimulated to do so. They also readily assumed phagocytic properties and became macrophages, or rounded up and changed into fibroblasts.

Taliaferro and Mulligan[7] described lesions of lymph nodes in malaria and in *Listerella monocytogenes* infection, similar to those seen in cat 80. This was strong evidence that these reactions were not specific to the disease but only to the nature of the stimulus.

SUMMARY

Examination of cats at intervals during the development of panleucopenia, at the height of the disease, and soon afterwards revealed that the primary reactions occurred in the lymph nodes and lymphoid tissue of the ileum and were soon followed by reactions in the mucosa of the intestine. The liver, pancreas, and kidney responded late in the disease and in them the resulting injury was sometimes greater than that found in the primary centers.

In the lymph glands, the initial response soon after infection was a draining off of lymphocytes from medullary spaces and cords, accompanied by severe phagocytosis of erythrocytes. The primary nodules did not change extensively during the course of the disease, but the secondary centers expanded slightly, developed inclusions, and later showed some hyaline material.

The mucosa in the terminal portion of the small intestine was slightly damaged before the process extended to the duodenum. In both, there was extreme destruction of villi and of the epithelium of the crypts of Lieberkühn.

Soon after the disease was established, the bone marrow became aplastic and nonfunctional. At the height of the disease, clear, rounded areas resembling fat occupied at least 70 per cent of the section. The apparent increase in the fat was a result of rapid atrophy of myeloid tissue by failure of maturation of the embryonic blood elements. The first day after recovery, when the maturation inhibitory factor of the virus was no longer present, there was a great increase in the number of cells in the bone-marrow reticulum. The spaces were widened by the proliferation of the embryonic blood elements and, by the fourth day, the bone marrow appeared normal.

References

[1]Lawrence, J. S., and Syverton, J. T.: Spontaneous Agranulocytosis in the Cat. Proc. Soc. Exptl. Biol. and Med., *38*, (1939): 914-918.

[2]Hammon, W. D., and Enders, J. F.: A Virus Disease of Cats, Principally Characterized by Aleucocytosis, Enteric Lesions, and the Presence of Intranuclear Inclusion Bodies. J. Exptl. Med., *69*, (1939): 327-352.

[3]Riser, W. H.: The Behavior of the Peripheral Blood Elements in Panleucopenia (Agranulocytosis) of the Domestic Cat. To be published.

[4]Lucas, A. M., and Riser, W. H.: Intranuclear Inclusions in Panleucopenia of Cats. Am. J. Path., *21*, (1945): 435-465.

[5]Lawrence, J. S., Syverton, J. T., Shaw, J. S., and Smith, F. P.: Infectious Feline Agranulocytosis. Am. J. Path., *16*, (1940): 333-354.

[6]Drinker, C. K., and Yoffey, J. M.: Lymphatics, Lymph, and Lymphoid Tissue. Harvard University Press. Cambridge, Mass., (1941): 168.

[7]Taliaferro, W. H., and Mulligan, H. W.: The Histopathology of Malaria with Special Reference to the Function and Origin of the Macrophages in Defense. Indian Med. Res. Mem., *29*, (1937): 1-128.

[8]Maximow, A. A., and Bloom, Wm.: A Textbook of Histology. 3rd ed. W. B. Saunders Co., Philadelphia, (1939): 48-107.

The Effect of Certain Chemical Agents on the Virus of Infectious Bronchitis of Chickens

CHARLES H. CUNNINGHAM, B.S., M.S., D.V.M., and H. O. STUART, B.S., M.S.
Kingston, Rhode Island

REPORTS OF THE EFFECT of certain chemical agents on the virus of infectious bronchitis of chickens are not available in the literature to serve as a possible means of differential diagnosis of this and other viruses producing respiratory diseases of fowl and the possible bearing upon disinfection in practice. The work of Dunham and MacNeal[1, 2] with the influenza and vaccinia viruses suggested comparative studies of the bronchitis virus.

Bronchitis virus when first isolated from chickens and inoculated into embryonating eggs fails to kill embryos for the first few passages. With each succeeding passage the virus becomes more virulent for embryos until finally the bulk of the embryos are killed by the end of the second day following inoculation, The ability of the virus to kill embryos appears to become fixed so that no further changes in this respect are noted.[3,4]

PROCEDURE

A strain of bronchitis virus which had been adapted to cultivation in embryonating chicken eggs was used for inoculum.* This virus was capable of killing the majority of 10- or 11-day embryos by the end of the second day and the remainder by the end of the fourth day. The virus suspension employed in the tests consisted of pooled allantoic fluid from embryos dead on the second day following inoculation with the virus *via* the allantoic sac route. An aliquot sample from the pooled virus suspension was used for each of the tests. Some tests were conducted with 10-day embryos and others with 11-day embryos but, in the tests, all embryos employed for a particular chemical agent were of the same age. The site of injection into the allantoic sac was determined by transillumination of the egg and selection of an area of the chorioallantoic membrane free of large blood vessels about 3 mm. below the base of the air cell. A short groove was drilled through the shell by means of a small carborundum disc on a mandrel attached to the chuck of an electric motor without piercing the shell membrane. Tincture of metaphen was painted over the groove in the shell and allowed to dry before the eggs were inoculated. Ten eggs were used for each test solution. After injecting the test mixture into the allantoic sac of each egg of the series, using a B-D Yale, 1-cc. capacity tuberculin syringe fitted with a 27-gauge, half-inch needle, the holes in the shells were sealed with melted paraffin and the eggs returned to the incubator. All incubation was at 99 F. in an electric, forced-draft incubator.

The test solutions were at room temperature when used, but the virus suspension was kept immersed in a water bath at 4 C. until mixed with the test solution. In all tests, 1 part of the virus supension was added to 9 parts of the test solution, thoroughly mixed, and allowed to stand for three minutes before injection in 0.05-cc. amounts into the eggs. In some instances, the mixture was injected into the eggs directly or after dilution with sterile distilled water to a chemical concentration which previous experiments had shown to be nontoxic for the embryo. In no instance was the virus diluted to such an extent as to materially influence its lethal action on the embryo.

After inoculations, the eggs were candled daily. Survival of the embryo for four days was considered as evidence that the material injected did not contain active virus.

The concentration of the stock virus suspension employed was determined by injecting 0.05 cc. of tenfold serial dilutions into eggs with the following results per dilution in which the denominator indicates the numbers of eggs inoculated and the numerator the numbers of embryos killed: 5×10^{-2} - 8/8, 10^{-3} - 8/8, 5×10^{-3} - 7/8, 10^{-4} - 7/8, 5×10^{-4} - 7/8, 10^{-5} - 4/8, and 5×10^{-5} - 1/8. The titer was considered to be the highest dilution which

Contribution No. 678 of the Rhode Island Agricultural Experiment Station, Kingston, R. I.

Present address of the senior author: Department of Bacteriology and Public Health, School of Veterinary Medicine, Michigan State College, East Lansing.

*Virus supplied by Dr. E. F. Waller, University of New Hampshire, Durham, N. H. Present address: University of Vermont, Burlington, Vt.

killed 50 per cent or more of the embryos inoculated. The titer was the 10^{-5} dilution and there were considered to be 10^5 embryo m. l. d.'s in 0.05 cc. of the original suspension.

Complete inactivation occurred with tincture of zephiran, 1 : 1,000; Lugol's solution, 1 per cent; sodium hydroxide, 1 : 20;

TABLE 1—Results of Inoculations of Eggs with Mixtures of Bronchitis Virus and Certain Chemical Agents (Reaction period 3 minutes. Inoculum 0.05 cc.)

	Concentration	Subsequent dilution of mixture	Mortality in 4 days
Phenol	3%	100	0/10
Phenol	1%	100	0/10
Liquor cresolis saponatus, U.S.P. XII	3%	100	0/10
Liquor cresolis saponatus, U.S.P. XII	1%	100	0/10
Tincture of metaphen		1,000	0/10
Tincture of metaphen	1%	10	0/10
Potassium permanganate	1:1,000	10	0/10
Potassium permanganate	1:10,000		0/10
Mercuric chloride	1:1,000	100	0/10
Mercuric chloride	1:10,000	10	1/10
Ethyl alcohol	95%		0/10
Ethyl alcohol	70%		0/10
Ethyl alcohol	40%		0/10
Ethyl alcohol	25%		0/10
Tincture of iodine, U.S.P. XII	0.1%	100	9/10
Tincture of iodine, U.S.P. XII	0.01%		10/10
Tincture of zephiran	1:1,000	100	0/10
Tincture of zephiran	1:100,000		10/10
Lugol's solution, U.S.P. XII	1.0%	100	0/10
Lugol's solution, U.S.P. XII	0.1%	10	10/10
Sodium hydroxide	1:20	500	0/10
Sodium hydroxide	1:10,000		10/10
Neoprontosil	5%	500	0/10
Neoprontosil	0.1%	10	10/10
Formalin	1.0%	500	0/10
Formalin	0.01%	10	10/10
Boric acid	4.0%	10	10/10

RESULTS

The results of the tests are recorded in table 1. The agents tested were per cent solutions with a few exceptions which were prepared as dilutions. Equivalent per cent solutions of the dilutions reported throughout the text and in table 1 would be as follows: 1 : 20—5 per cent, 1 : 1,000—0.1 per cent, 1 : 10,000—0.01 per cent, 1 : 100,000—0.001 per cent. The following agents completely inactivated the virus during the three-minute reaction period: phenol, 3 per cent and 1 per cent; liquor cresolis saponatus, 3 per cent and 1 per cent; tincture of metaphen, undiluted and 1 per cent; potassium permanganate, 1 : 1,000 and 1 : 10,000; and ethyl alcohol, 95 per cent, 70 per cent, 40 per cent, and 25 per cent. The tests with mercuric chloride gave results almost identical with potassium permanganate.

Neoprontosil, 5 per cent; and formalin, 1 per cent, but these same antiseptics were without effect in the following dilutions: tincture of zephiran, 1 : 100,000; Lugol's solution, 0.1 per cent; sodium hydroxide, 1 : 10,000; Neoprontosil, 0.1 per cent, and formalin, 0.01 per cent.

Boric acid in a 4 per cent solution was without effect. Tincture of iodine in a 0.1 per cent solution produced a slight inactivation but in a 0.01 per cent solution it was without effect.

DISCUSSION

The results obtained in the present study of the bronchitis virus and those of Dunham and MacNeal[1, 2] for the vaccinia and influenza viruses show several interesting variations on the effect of the agents on the viruses under similar test conditions. The influenza virus was almost completely

inactivated by a 3 per cent solution of phenol which was moderately effective against vaccinia virus while a 1 per cent solution had but little effect on the influenza virus and was without effect on the vaccinia virus. The bronchitis virus was completely inactivated by both dilutions of phenol. Potassium permanganate, 1 : 1,000, failed to inactivate the vaccinia virus. The influenza virus was almost completely inactivated by the 1 : 1,000 dilution, but in the 1 : 10,000 dilution no effect was observed. The bronchitis virus was completely inactivated by both dilutions of potassium permanganate. Ethyl alcohol, 40 per cent, inactivated the influenza virus to an extent that slightly less than half of the inoculated embryos succumbed to the action of the virus, but a 25 per cent solution had no effect on either the influenza or vaccinia virus. The bronchitis virus was completely inactivated by both dilutions of ethyl alcohol. Mercuric chloride, 1 : 1,000 dilution, almost completely inactivated the influenza virus and was moderately effective against the vaccinia virus, but a 1 : 10,000 dilution produced slight inactivation of the influenza virus and no inactivation of the vaccinia virus. With the bronchitis virus, complete inactivation occurred with the 1 : 1,000 dilution and, in the 1 : 10,000 dilution, the inactivation was nearly complete. Tincture of iodine, 0.1 per cent solution, failed to inactivate the vaccinia virus. The influenza virus was almost completely inactivated by a 0.1 per cent solution but a 0.01 per cent solution was without effect. With the bronchitis virus, there was only a slight effect produced by a 0.1 per cent solution and no effect by a 0.01 per cent solution. The influenza and bronchitis viruses gave identical results with Lugol's solution: complete inactivation in a 1 per cent solution but no inactivation in 0.1 per cent solution. Boric acid, 4 per cent solution, was without effect against the influenza and bronchitis viruses but produced a slight inactivation of the vaccinia virus.

Selection of comparative tests in which the viruses were either completely inactivated or in which the agents were without effect indicated the following: phenol, 1 per cent, bronchitis-inactivated, no effect on vaccinia; potassium permanganate, 1 : 1,000, bronchitis-inactivated, no effect on vaccinia, 1 : 10,000, bronchitis-inactivated, no effect on influenza; ethyl alcohol, 25 per cent, bronchitis-inactivated, no effect on influenza or vaccinia; tincture of iodine, 0.01 per cent, no effect on bronchitis and influenza; Lugol's solution, 1 per cent, inactivation of bronchitis and influenza but no effect by a 0.1 per cent solution; boric acid, 4 per cent, no effect on bronchitis or influenza.

These experiments were conducted with an egg-propagated strain of the bronchitis virus and it was impossible to predict what comparative results might be obtained with chicken-propagated virus, using live chickens for inoculations. Since laboratory studies of the bronchitis virus must, of necessity, utilize an egg-propagated strain, it is hoped that the results presented in this contribution may stimulate experimentation to gain further knowledge of the virus and possible related strains and their relationship to other viruses as a means of more accurate differential diagnosis of the virus diseases of animals.

SUMMARY

A number of chemical agents were tested for their effect on an egg-propagated strain of the virus of infectious bronchitis of chickens during an exposure period of three minutes. Following the exposure period, the virus-chemical mixture was injected into the allantoic cavity of embryonating chicken eggs. Some of the mixtures were injected into the eggs directly while others were diluted with sterile distilled water to a chemical concentration which previous experiments had shown to be nontoxic for the embryo. Survival of the inoculated eggs for four days was considered as evidence that the material injected did not contain active virus.

The following preparations inactivated the virus in three minutes or less: phenol, 3 per cent and 1 per cent; liquor cresolis saponatus, 3 per cent and 1 per cent; tincture of metaphen, undiluted and 1 per cent; potassium permanganate, 1 : 1,000 and 1 : 10,000; mercuric chloride, 1 : 1,000; ethyl alcohol, 95, 70, 40 and 25 per cent; tincture of zephiran, 1 : 1,000; Lugol's solution, 1 per cent; sodium hydroxide, 1 : 20; Neoprontosil, 5 per cent; and formalin, 1 per cent. The following preparations were without effect during the three-minute exposure period: tincture of iodine, 0.01 per cent; tincture of zephiran, 1 : 100,000; Lugol's solution, 0.1 per cent; sodium hydroxide, 1 : 10,000; Neoprontosil, 0.1 per cent; formalin, 0.01 per cent; and boric acid, 4 per cent.

References

[1]Dunham, W. B., and MacNeal, W. J.: Inactivation of Vaccinia Virus by Mild Antiseptics. J. Lab. and Clin. Med., *28*, (1943): 947-953.

[2]Dunham, W. B., and MacNeal, W. J.: Inactivation of Influenza Virus by Mild Antiseptics. J. Immunol., *49*, (1944): 123-128.

[3]Beaudette, F. R. and Hudson, C. B.: Cultivation of the Virus of Infectious Bronchitis. J. A. V. M. A., *90*, (1937): 51-58.

[4]Delaplane, J. P., and Stuart, H. O.: The Modification of Infectious Bronchitis Virus of Chickens as the Result of Propagation in Embryonated Chicken Eggs. Rhode Island Agric. Exper. Sta., Bul., 284, 1941.

Reaction of the Developing Chicken Embryo and the Chorioallantoic Membrane to Inoculation with Various Strains of Mycobacterium Paratuberculosis (Johne's Bacillus)

ABRAM B. STAVITSKY, Ph.D., V.M.D., and JOHN D. BECK, V.M.D.
Philadelphia, Pennsylvania

EXPERIMENTAL STUDIES of paratuberculosis or Johne's disease have been hampered by the lack of a suitable experimental host other than the cow, sheep, and goat. Numerous attempts have been made to infect small laboratory animals, but no disease simulating the natural enteric infection has been consistently produced in any animal other than ruminants[1]. Therefore, in studies aimed at determining the chemotherapeutic value of various drugs against *Mycobacterium paratuberculosis*, the need arose for a method of producing a suitable consistent experimental infection. The local lesions produced at the point of injection of large doses of those organisms into any animal[2], or the tubercle-like masses which appear on the great omentum following intraperitoneal injection of the bacilli[2], did not seem to answer this purpose. In view of the use of the chicken embryo for the cultivation of many diverse microörganisms, including other pathogenic acidfast bacilli[3, 4, 5, 6], it was considered for this study. In the only study of its type found in the literature, five- and ten-day chicken embryos were inoculated with large doses of *M. paratuberculosis* suspended in mineral oil and the chickens produced were intradermically tested at the age of 3 months[7]. As these embryos were not studied during embryonic life, no conclusions can be drawn regarding their adaptability for chemotherapeutic studies. In this paper, the results of the inoculation by various routes, with various strains of *M. paratuberculosis* are presented. The applicability of the embryo to the study of chemotherapeutic agents against this and other organisms is discussed. Finally, the use of various aspects of the chicken embryo infection in furthering our understanding of the natural disease is considered.

From the School of Veterinary Medicine, University of Pennsylvania, Philadelphia. Aided by a grant from the Faculty Research Committee of the University of Pennsylvania.

Dr. Stavitsky is now at the Gates and Creelin Laboratories of Chemistry, California Institute of Technology, Pasadena.

MATERIALS AND METHODS

The strains of *M. paratuberculosis* used, and their histories, are summarized below. The most significant point about these strains is that all but one, the Wisconsin—strain M., required the addition of dead cells of *Mycobacterium phlei* to the medium to secure growth.

1) From Dr. Elizabeth McCoy, College of Agriculture, University of Wisconsin—strain M. This strain was isolated about 1927 by Hastings, Mansfield, and Beach[8] from a cow infected with this organism. It has always been comparatively easy to cultivate in the laboratory, not requiring the cells of *M. phlei*, or vitamin K[9], the special growth factor for this organism[10].

2) From Dr. B. T. Simms, formerly director, Regional Animal Disease Laboratory, Bureau of Animal Industry, Auburn, Ala. (*a*) Strain Peto—isolated May 11, 1924, by the Medical Research Council, England. (*b*) Strain 41—isolated Oct. 15, 1943, by Dr. E. P. Johnson at the Virginia Agricultural Experiment Station. (*c*) Strain 157—isolated May 28, 1942, by Medical Research Council, England, from a very early intestinal lesion of Johne's disease. (*d*) Strain 167—isolated May 16, 1943, by Medical Research Council, England.

3) From Dr. E. P. Johnson, Virginia Agricultural Experiment Station, Blacksburg, Va.: (*a*) Strain V—isolated December, 1943, from a case of Johne's disease. (*b*) Strain C 99—a recently isolated strain used by Dr. Johnson in attempts to infect cats and hamsters.

4) From Dr. A. Zeissig, New York State Veterinary College, Ithaca, N. Y.: (*a*) Cornell Strain—recently isolated. (*b*) Promise Strain—recently isolated.

These strains were maintained regularly on the bovine lymph gland medium described by Johnson[11]. Abundant growth was obtained in from three to four weeks on this medium.

Suspensions of known numbers of bacilli were prepared as follows: 3- or 4-week-old cultures were used. The dry growth of bacilli was weighed on a sheet of platinum foil which had previously been flamed and allowed to cool. After preliminary emulsification in a drop of ox bile, the weighed bacilli were thoroughly ground in physiologic saline in an agate mortar with a pestle. The suspension was then filtered through a Whatman 5 filter paper. All solutions and equipment were sterile. The filtrate was adjusted to contain the desired number of organisms by counting the bacilli in a Petroff-Hauser counting chamber by means of dark field illumination, and by diluting the filtrate to the desired concentration with normal saline. One milligram of dry growth contained approximately 100 million organisms although this figure varied from strain to strain, being as high as 500 million for several strains and as low as 50 million for other strains.

Unless otherwise indicated, eggs of eleven days' incubation were inoculated. The technique of injection of the eggs, which is described in detail elsewhere[12], was briefly as follows: The shell over the air sac was removed, and the shell membrane stripped from the underlying chorioallantois. The embryonic sacs were then clearly seen, and selective or combined intravenous, intrayolk, intra-amnionic or intra-allantoic injections or aspirations accurately made; 0.05 cc. of fluid can be inoculated into very small allantoic venules. The open end of the egg was sealed with two layers of Scotch tape through which the embryo was visible, permitting ready determination of its death, without candling.

After inoculation, the eggs were incubated at 37.5 C. Usually after nine to ten days, when the eggs were 20 to 21 days old, they were opened, the embryos removed, and various tissues saved for cultural and histologic studies. For histologic studies, the tissues were fixed in 10 per cent formalin, embedded in paraffin, and sectioned. The sections were stained for the acidfast bacilli by the Ziehl-Neelsen method, and were counter stained with hematoxylin to bring out nuclear detail. Duplicate slides were stained with hematoxylin and eosin to study the nature of any lesions present.

All strains of the bacilli were inoculated into the embryos by the following routes, on the chorioallantoic membrane, into the allantoic cavity, amnionic cavity, yolk sac, and into an allantoic venule. The amount of each strain injected by each route was as follows:

1) On the chorioallantois, 0.25 mg. of strain 167, 0.05 mg. of other strains.

2) Into the allantoic cavity, 0.1 mg. of strains 41 and Peto, 0.5 mg. of the other strains.

3) Into amnionic cavity, 0.5 mg. of each strain.

4) Intravenously, 0.05 mg. of strains Peto and 41, 0.5 mg. of other strains.

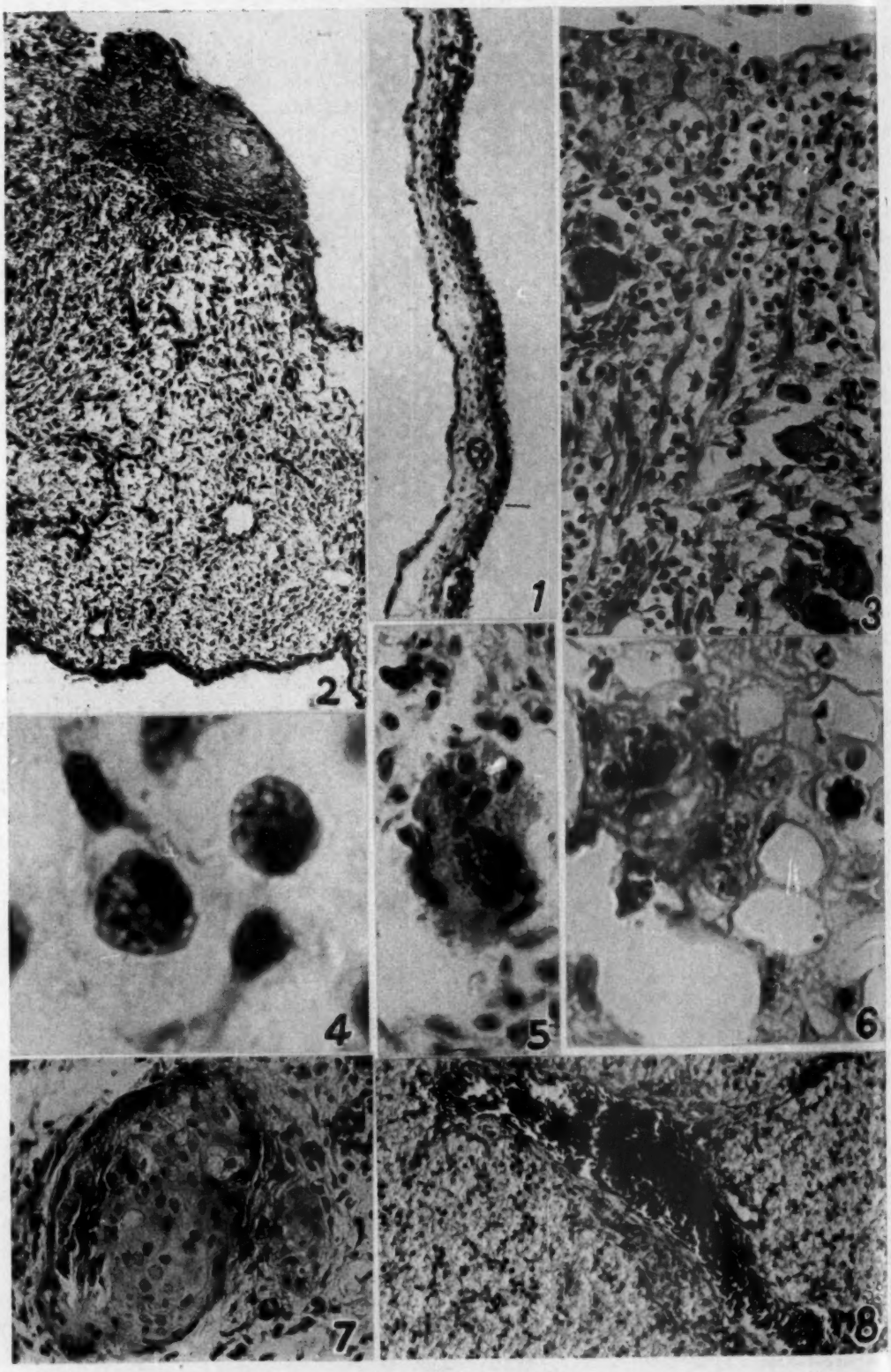
5) Into yolk sac; 0.1 mg. strains Peto and 41, 0.5 mg. of other strains. Usually 18 eggs constituted a group. In every experiment, an equivalent number of eggs was opened but not injected to serve as controls, and another group included eggs injected with the sterile saline diluent only.

EXPERIMENTAL DATA

Survival of Eggs.—In several experiments, due to the poor viability of the eggs used, as judged by the poor survival of the controls, no accurate data on the survival of the infected eggs could be obtained. However, in most instances, this was possible. Typical results of experiments are summarized in table 1.

As may be seen from the table, 11-day embryos withstood the opening procedure and injection of saline fairly well, particularly inoculation on the chorioallantoic membrane, into the yolk sac, and allantoic cavity. The survival percentage of the eggs injected with the organisms was sufficiently different from the survival of the control eggs to permit the use of survival as a possible criterion of the efficacy of various chemotherapeutic agents against any infection which might develop following injection. In experiments in which 12- and 13-day embryos were used, the survival in all groups was much better than in the younger eggs, making older eggs more suitable for chemotherapeutic studies, if enough time for development of the infection is afforded by use of the older eggs. Further data on the survival of eggs injected by this technique are given in another paper[12].

Result of Inoculation of Embryos with Strains of M. paratuberculosis other than Strain M.—None of these strains produced


1
2
3
4
5
6
7
8

an infection in the chorioallantoic membrane of the embryo using positive cultures and the presence of acidfast bacilli in tissue sections as criteria. Grossly, the membranes were thicker than the control membranes and somewhat cloudier. However, there were extensive histologic changes in some of the tissues from these embryos. As these changes were about the same regardless of the strain used, they will be presented in the form of a general description.

Figure 1 shows the normal chorioallantoic membrane of the chicken embryo at the time of hatching. The membrane consists of an ectodermal layer of two rows of cells. Lying directly beneath the outer epithelial row is the mesodermal row composed largely of connective tissue through which the blood vessels extend. The tissue of this layer is composed of a loose meshwork of mesenchymal cells, polygonal and spindle shaped. The under surface of the mesodermal tissue is lined with a row of flat pavement cells.

Figure 2 illustrates the response of the chorioallantois to the deposition of Johne's bacilli on it. The membrane is greatly thickened, mainly by edematous infiltration, as is shown by the pushing apart of the fibroblast-like mesodermal cells. There are both diffuse and focal, nodular proliferations of ectodermal cells. There is a great proliferation of the mesenchymal cells as well. Many mononuclear and polymorphonuclear cells may be seen in clusters or spread through the mesodermal layer. Many of the mononuclear cells have a vacuolated cytoplasm, suggestive of dissolved lipoidal material (fig. 4). Not shown in the illustrations is the proliferation of new capillaries, also in the mesodermal layer. In some membranes, the ectoderm was so hyperplastic that the uppermost layers became eosinophilic and sloughed off much as do the upper keratinized layers of mammalian skin. Occasionally, islands of epithelial cells were detached from the surface epithelium as "epithelial pearls" (fig. 7). The blood vessels of the membranes were often engorged with predominantly mononuclear cells. There was no trace of acidfast bacilli in any of these membranes. No changes were seen in the control membranes.

After inoculation of the organisms into the amnionic cavity, allantoic cavity, yolk sac, or venules of the embryos, and the elapse of a suitable incubation period, the following tissues were saved for examination; yolk sac, chorioallantoic membrane, liver, spleen, glandular stomach, and small intestine. Acidfast bacilli were not observed in any of these tissues. There were

Legends for Illustrations on Opposite Page

Fig. 1—Normal chorioallantois of 21-day chicken embryo. x 150. Compare with fig. 2 which is of same magnification.

Fig. 2—Chorioallantois of 21-day chicken embryo on which Johne's bacilli had been deposited. x 150. Note edematous thickening of membrane and nodular proliferation of ectodermal cells which may be seen in upper right hand corner of illustration. There is a great proliferation of mesodermal cells. Many mononuclear and polymorphonuclear cells are spread throughout the mesoderm. Compare with fig. 1 of same magnification.

Fig. 3—Villus of small intestine from cow naturally infected with Johne's bacilli. x 300. Lumen is light area at top of illustration. Villus is thickened and filled with granulation tissue. The latter consists of epithelioid cells, plasma cells, giant cells with peripheral nuclei, lymphocytes, polymorphonuclear cells, and some eosinophils. Compare the giant cells with those in chorioallantois in fig. 5.

Fig. 4—Mononuclear or young epithelioid cells in chorioallantois of chicken embryo infected with Johne's bacilli. x 2130. Note vacuolated cytoplasm.

Fig. 5—Giant cell in chorioallantois of chicken embryo infected with strain M of Johne's bacillus. x 620. Compare with giant cells in natural disease in fig. 3. Note peripheral nuclei in this cell and the clump of bacilli in the cytoplasm within the nuclei.

Fig. 6—Clumps of Johne's bacilli, strain M, in chorioallantois of chicken embryo. x 660. These bacilli were found in the mesoderm of the membrane.

Fig. 7—Epithelial pearl in mesoderm of chorioallantois of chicken embryo infected with Johne's bacilli. x 300.

Fig. 8—Liver of 21-day chicken embryo injected with Johne's bacilli. x 150. Note uniform infiltration of hepatic cords by fat globules, infiltration of mononuclear cells between some of the hepatic cords.

no gross or microscopic changes in any of the tissues except the liver. In all embryos, the liver showed extreme fatty metamorphosis, cuffing of many of the vessels with mononuclear cells (fig. 8). In some livers in which not all of the parenchyma was fatty, the hepatic cords were dissociated, and mononuclear cells occupied the spaces between the cords. No changes were seen in the livers of uninoculated embryos or embryos injected with saline.

Results of Inoculation of Embryos with Strain M.—Upon deposition of strain M. on the chorioallantoic membrane, infection of the membrane was consistently obtained. Positive cultures were obtained from the membranes at all stages of the incubation period. Multiplication of the bacilli could be clearly demonstrated in histologic sections. Few bacilli were seen in membranes removed within a day of injection, whereas, numerous organisms, mainly in clumps and extracellular, were observed in the mesenchyma of membranes removed from the embryos just before hatching (fig. 6). With a few exceptions, the changes in these membranes resembled those seen after injection with other strains of Johne's bacillus, but were much more intensive and extensive. Giant cells containing peripheral nuclei enclosing clumps of bacteria (fig. 5) were seen in every membrane. There were focal accumulations of mononuclear cells, heterophilic polymorphonuclears, and lymphocytes. There were tremendous proliferations of ectodermal cells and mesenchymal cells and some epithelial pearls as seen before (fig. 2 and 7). The blood vessels were engorged with mononuclear cells with vacuolated cytoplasm (fig. 4). In general, these changes were reminiscent of those in the natural disease.

Figure 3 is an illustration of a villus from the small intestine of a cow infected with Johne's disease. The villus is thickened and filled with granulation tissue consisting of epithelioid cells, plasma cells, giant cells with peripheral nuclei (compare with fig. 5) lymphocytes, polymorphonuclear cells, and some eosinophils.

Within the embryo, the changes produced by inoculation of strain M by routes other than on the membrane were indistinguishable from those produced by the other strains inoculated by these routes. Fatty metamorphosis of the liver was the predominant change (fig. 8). However, in contradistinction to the membrane, multiplication of the organisms was not obtained within the embryo.

Non-Acidfast Forms of M. paratuberculosis.—In several of the membranes inoculated with strain M. non-acidfast forms of *M. paratuberculosis* were seen. These forms took the methylene blue counterstain. They were observed in the interior of globular structures to the periphery of which the usual acidfast forms were attached. The

TABLE I—Survival Data on Chicken Embryos Infected with Mycobacterium Paratuberculosis

Strains of Bacillus	Age of Embryo	Dose (mg.)	Route Inoc.	Survival Controls %	Survival Saline Inj. %	Survival Infected Eggs %
M	11 days	0.5	C.A.	12/18= 67	12/18= 67	4/18= 22
Promise	11 days	0.5	C.A.	14/18= 78	13/18= 70	5/18= 28
Cornell	11 days	0.5	C.A.	16/18= 90	13/18= 70	3/20= 15
167	11 days	0.25	C.A.	16/19= 80	14/18= 78	4/18= 22
M	9 days	0.5	I Am.	8/11= 73	8/11= 73	3/11= 27
M	11 days	0.5	I V	8/11= 73	8/11= 73	2/20= 10
M	11 days	0.5	I Al.	8/11= 73	8/11= 73	8/11= 73
M	11 days	0.5	I Y	8/11= 73	8/11= 73	4/11= 36.4
Peto	11 days	0.05	I V	6/12= 50	6/12= 50	1/12= 8
Peto	11 days	0.1	I Y	6/12= 50	6/12= 50	8/20= 40
Peto	11 days	0.1	I Al.	6/12= 50	6/12= 50	7/11= 63
41	11 days	0.05	I V	8/12= 75	8/12= 75	2/10= 20
41	11 days	0.1	I Y	8/12= 75	8/12= 75	3/11= 63
41	11 days	0.1	I Al.	8/12= 75	8/12= 75	5/12= 41

Route inoculation—C.A.= chorioallantois; I Am.= intra-amnionically; IV= intravenously; I Al.= intra-allantoically; IY= intrayolk sac.

Survival—numerator= number of survivors; denominator= number injected or opened.

non-acidfast forms were granular in appearance as distinguished from the short rod forms of the acidfast organisms.

DISCUSSION

Using positive cultures and the demonstration of the multiplication of acidfast bacilli in tissue sections as criteria, infection with *M. paratuberculosis* was achieved only in one experiment, that in which Strain M was applied to the chorioallantois. It is of interest that this strain of organism was the only one which did not require the addition of *M. phlei* for growth. The question might be raised whether the addition of dead cells of *M. phlei* to the inoculum of *M. paratuberculosis* would enable the "fastidious" strains of this organism, which require *M. phlei*, to multiply on the chorioallantois. In several experiments in which this was done, the results were irregular, multiplication of Johne's bacilli occurring in one experiment and not in the others. This question is certainly worthy of further investigation. However, from the data presented, it is certain that Strain M of Johne's bacillus, and possibly other "non-fastidious" strains of this organism not requiring the *M. phlei* factor for growth on artificial mediums, may be used for the pilot testing of chemotherapeutic agents against this organism on the chorioallantoic membrane. The technique and its application to the screening of chemotherapeutic agents against the human tubercle bacillus are presented elsewhere[12, 13].

It is of great interest for an understanding of the histogenesis of the lesions in the natural disease in the bovine animal to consider the changes produced in the embryo and the chorioallantoic membrane in the absence of multiplication of the bacilli. Though less marked than the changes seen in the presence of multiplication of strain M, the changes in the chorioallantois are predominantly proliferative in nature. It is realized that this membrane responds to a variety of irritants with proliferation of its cellular elements. However, the presence of mononuclear cells with vacuolated cytoplasm is especially significant. These cells strongly resemble the cells called young epithelioid cells which were produced by the injection into guinea pigs and rabbits of lipoid fractions of various types of tubercle bacilli[14]. It is known that Johne's bacilli are rich in lipoid. Extensive lesions in the small intestine of the bovine animal, including many young and mature epithelioid cells, and serious symptoms are sometimes observed without demonstrable bacilli. We suggest that this may be considered a reaction to dissolved lipoids and possibly other chemical fractions of the bacilli. The acute inflammation sometimes seen in the small intestine of the bovine animal was also noted in the membranes and testifies to the intense irritant nature of the organisms and their products. The fatty degeneration of the embryonic liver is further evidence of the toxic nature of the organisms and their products as this change was produced in the absence of multiplication of the bacilli.

There was little evidence of phagocytosis of the bacilli as is seen in the natural disease. The formation of giant cells in the presense of multiplying bacilli could be readily traced in some sections. There was no doubt that these cells were formed by the fusion of mononuclear cells around the organisms.

Another reason for the lack of correlation between the intensity of the symptoms and tissue changes and the demonstration of acidfast bacilli in the natural disease is the possibility that non-acidfast forms of *M. paratuberculosis* might be unnoticed in the area. Jackson has recently demonstrated, by means of a special stain, such non-acidfast form of human tubercle bacilli in material from cases of human tuberculosis[15]. Therefore, it might be worth while to use this special stain on material from natural cases of Johne's disease in an attempt to find non-acidfast forms of the organism.

Finally, this study and many others which have gone before it demonstrate the suitability of the chicken embryo to the solution of diverse problems, including the

fields of chemotherapy of infections and experimental pathology.

SUMMARY

1) Strain M, a strain of Johne's bacillus not requiring *Mycobacterium phlei* for growth on artificial mediums, produced an infection of the chorioallantoic membrane of the developing chicken embryo, characterized by extensive multiplication of the bacilli, proliferation of the ectoderm and mesoderm of the membrane, and infiltration of mononuclear, polymorphonuclear, and giant cells into the mesoderm. The same organisms, injected into the the embryo by various routes, produced fatty metamorphosis of the liver but did not multiply anywhere within the embryo.

2) A number of other strains of Johne's bacillus, all requiring *M. phlei* for growth, did not multiply anywhere on the membrane or in the embryo, but produced changes differing only in their lesser intensity and the absence of giant cells in the membrane from those produced by strain M.

3) Non-acidfast forms of Johne's bacillus were found in several of the membranes which were injected with strain M. It is suggested that animal pathologists look for this form of the organism in material derived from the natural disease as a possible explanation of the lack of correlation between the intensity of the lesions and number of bacilli found in them.

4) The applicability of this technique, injection of a nonfastidious strain of Johne' bacillus, to the chemotherapeutic testing of compounds against this organism was discussed.

5) The histogenesis of the lesions of the natural disease in terms of dissolved products, predominantly lipoids of the organisms, is discussed. This was adduced as a further possible explanation of the lack of correlation between numbers of organisms and intensity of lesions.

6) The suitability of the chicken embryo for studies in experimental pathology was again demonstrated.

ACKNOWLEDGMENT

The authors are very grateful to the following individuals for aid in carrying out this work: to Mrs. Ruth Stavitsky and Dr. Bernard Gabriel for technical assistance in certain phases of the work; to Dr. Evan L. Stubbs of the School of Veterinary Medicine, University of Pennsylvania, for furnishing the slide of the natural disease and for a stimulating discussion of certain features of the work; to Dr. Max B. Lurie of the Henry Phipps Institute, University of Pennsylvania, for verifying the presence of non-acidfast forms of *M. paratuberculosis* in the chorioallantoic membranes.

References

[1]Hagan, W. A., in The Mycobacterial Diseases, Symposium Series, Vol. 1, of The American Association for the Advancement of Science, (1938): 69-79.

[2]Hagan, W. A.: The Infectious Diseases of Domestic Animals, Comstock Publishing Co., Ithaca, N. Y., 1943.

[3]Goodpasture, E. W., and Anderson, K.: The Problem of Infection as Presented by Bacterial Invasion of the Chorio-Allantoic Membrance of Chick Embryos. Am. J. Path., *13*, (1937): 149-174.

[4]Costil, L., and Bloch, F.: Réactions de la membrane chorio-allantoïde de l'embryon de poult aux bacilles tuberculeaux humains et aviaires. Compt. rend. Soc. Biol., *128*, (1938): 40-42.

[5]Moore, M.: The Chorio-allantoic Membrane of Chick Embryos and its Response to Inoculation with Some Mycobacteria. Am. J. Path., *18*, (1942): 827-847.

[6]Emmart, E. W., and Smith, M. I.: The Growth and Effects of the Tubercle Bacillus on the Chorio-allantoic Membrance of the Chick Embryo: A Method for Studies in Chemotherapy. Pub. Health Rep., *56*, (1941): 1277-1286.

[7]Johnson, H. W., and Cox, B. F.: Studies on Johnin. I. The Comparative Sensitivity of Rabbits, Guinea Pigs and Chickens Inoculated with Mycobacterium paratuberculosis. Am. J. Vet. Res., *3*. (1942): 131-138.

[8]Hastings, E. G., Beach, B. A., and Mansfield, H. L.: Johne's Disease, A Transmissible Disease of Cattle. Univ. of Wis. Agric. Exper. Sta. Bull., 81, December, 1927.

[9]McCarter, J. R.: Personal communication.

[10]Wooley, D. W., and McCarter, J. R.: Antihemorrhagic Compounds as Growth Factors for the Johne's Bacillus. Proc. Soc. Exptl. Biol. Med., *45*, (1940): 357-359.

[11]Johnson, H. W.: Studies on Johnin. V. Producing and Standardizing a Potent Product. Am. J. Vet. Res., *5*, (1944): 320-328.

[12]Lee, H. F., Stavitsky, A. B., and Lee, M. P.: A Chick Embryo Technic for Intravenous and Chemotherapeutic Studies. Proc. Soc. Exptl. Biol. Med. In Press.

[13]Lee, H. F., Stavitsky, A. B.,: Infection of the Chick Embryo with the Human Tubercle Bacillus. To be published.

[14]Smithburn, K. C., and Sabin, F. R.: The Cellular Reactions to Lipoid Fractions from Acidfast Bacilli. J. Exptl. Med., *56*, (1932): 867-892.

[15]Alexander-Jackson, E.: A Differential Stain for Demonstrating and Studying Non-Acid-Fast Forms of the Tubercle Bacillus in Sputum, Tissue, and Body Fluids. Science, *99*, (1944): 307-308.

Two Examples of Terata in the Caudal Region of Domestic Poultry

I. L. KOSIN, M.S.A., Ph.D.

Ottawa, Canada

RECORDED ABNORMALITIES involving both the urogenital and the alimentary systems of the domestic poultry are rare. Only two have come to the attention of the author: Crew[1] and Ottoway and Black[2] described clinical cases of hens with two vents. In both instances, the supernumerary structure was concerned only with urination and oviposition; there was no connection between the second vent and the intestine.

Such terata pose some interesting morphogenetic problems as they involve the questions of induction, organizers, and tissue potency. Hence, when 2 additional cases came to the attention of the author, it was deemed advisable to record and discuss them in the light of our present understanding of the phenomenon of morphogenesis in poultry.

First Case.—This was a Small White turkey hen hatched at the Central Experimental Farm in 1944. At hatching, the poult was seen to have a small, pear-shaped mass attached just ventrally to the coccyx. The mass, firm to the touch, had a normal cutaneous covering. There were two anal openings located just below the point of attachment of the mass, symmetrically placed on each side of the imaginary median line bisecting the body. Both openings were patent.

The poult, which weighed 0.12 lb. at hatching, was raised together with the other poults of the same age and breeding. The subsequent history of the bird was uneventful: it grew normally and when killed, at 26 weeks of age, weighed 8.10 lb., just slightly below pen average.

The pear-shaped mass was found to have grown considerably, measuring 55 mm. in length, 110 mm. in circumference at its widest part (which was near its posterior end), and 95 mm. in circumference at the narrow, anterior end. The skin covering was devoid of feathers, though a few feather follicles were scattered over the area. The anal openings were small, each about half as large in circumference as the single opening found in turkeys of comparable age and weight.

Fig. 1—Dissection of the posterior region (first case) showing the "pear-shaped mass". The indicator shows the point of juncture at the two short colonic passages. Note (arrows) the two cloacas.

Dissected from the body, the mass weighed 45.7 Gm. There was no bone connection between it and the rest of the body, the mass being suspended by the combination of fascia bridge and skin envelope. On dis-

From the Poultry Division, Central Experimental Farm, Ottawa, Canada. The specimens were made available for study through the cooperation of Messrs. H. I. MacGregor and J. L. Roy of the Poultry Division and of Dr. A. B. Wickware of the Poultry Pathology Division, Ottawa.

The author is now in the Department of Poultry Husbandry, State College of Washington, Pullman.

section, the mass was found to have, besides the already mentioned tissues, a layer of fatty tissue enveloping an osseous capsule which measured about 45 mm. in length and 70 mm. in circumference. The capsule was completely infiltrated with fatty tissue. No distinctly muscular tissue was identified anywhere in the capsule. The blood circulated *via* a branch of the coccygeal artery and a branch of the coccygeal vein.

Both the urinary and the alimentary systems were abnormal. Each of the two anal openings led into a small cloaca which was joined to a short (10 mm. long) narrow colonic canal (fig. 1). The two canals joined into one which, at the point of juncture, measured 20 mm. in diameter and then gradually narrowed, over a distance of 143 mm., down to 17 mm.

At the latter point were found three large ceca (fig. 2) each measuring about 240 mm. in length. The ceca were arranged symmetrically, *i.e.*, one on each side of the intestine and the third on the median ventral side. All three ceca opened into the lumen of the large intestine. Immediately cephalad to the point of juncture, the intestine narrowed down to about 8 mm. in diameter.

There was one bursa Fabricii which opened into the anterior portion of the left proctodeum. There were two left ureters. One of these, upon emerging from the kidney, split into two branches which however, before joining the urodeum, again fused into a single duct. The single left functional oviduct led into the left cloaca. The rudimentary right oviduct and the right ureter also joined the left cloaca.

Second Case.—This was a New Hampshire cockerel found in the flock of a Quebec poultryman and sent to the Poultry Division by Rev. Father Eloi Gerard of College

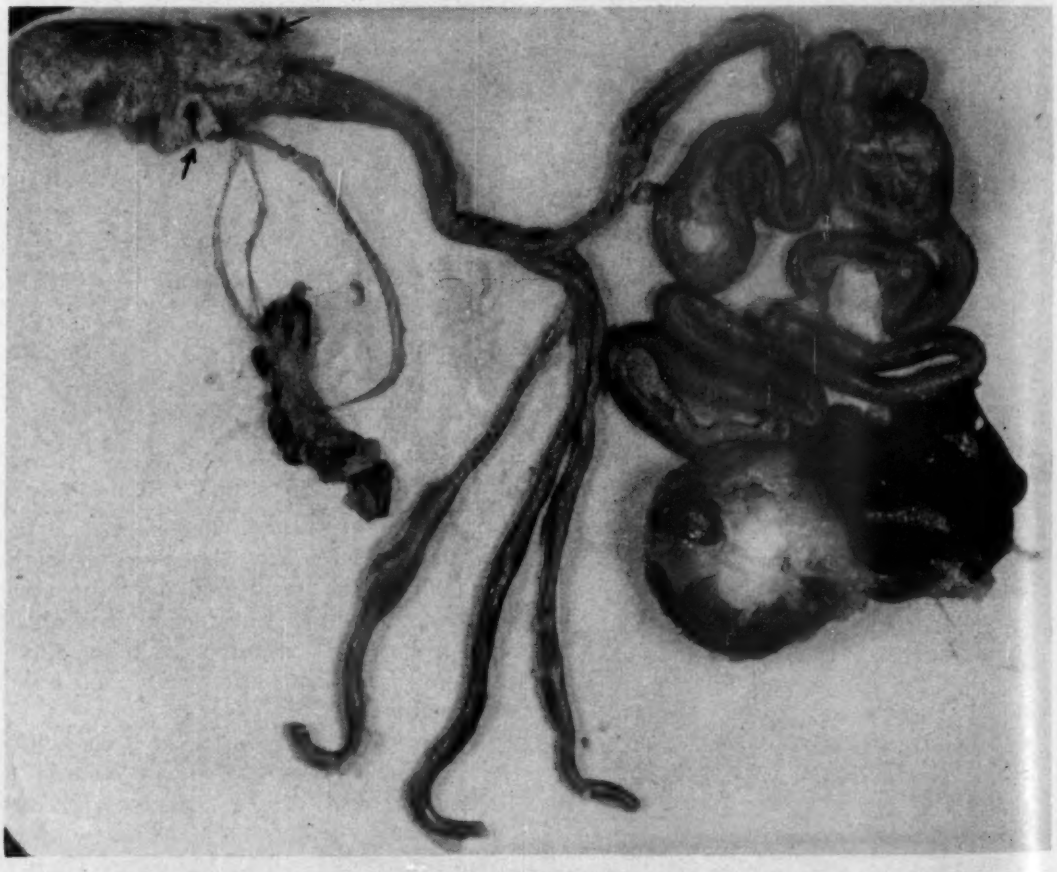


Fig. 2—Dissected alimentary and urinary systems (first case). Note three cecums and two left ureters, of which one is split. Arrows point to the cloacas.

du Sacre-Coeur, Beauceville Quest, Quebec. Information regarding the male was scanty: it hatched in March, 1945, and died sometime in December of the same year.

Upon superficial examination, the carcass was found to be that of a healthy male which had two anal openings symmetrically located on each side of a line parallel to the median plane (fig. 3). As in the first case, each opening was only half as large in circumference as is found in birds of comparable age. On dissection, the two openings were seen to lead into a common colon by two short and narrow colonic passages. The single colon was considerably larger than that found in normal birds of comparable age. The colon was impacted with feces. It appeared that the caudal colon, because of bifurcation, failed to cope with the problem of fecal evacuation. This led to gradual accumulation of feces in the colon, the condition which undoubtedly finally caused the death of the bird. The hypertrophy of the colon without doubt was in response to mechanical pressure exerted by the feces.

The urogenital system appeared to be normal. Dead spermatozoa were identified both in the vasa deferentia and the testes.

DISCUSSION

Lacking specific experimental data, one can only discuss the nature of the possible

Fig. 3—Full view of the caudal region in the New Hampshire male provided with two cloacas (second case).

forces responsible for the phenomenon which is the subject of this paper.

The anus in the gallinaceous birds is derived from the proctodeal invagination which, of course, is ectodermal. The invagination forms no direct connection either with the urodeal or coprodeal portions of the cloaca until shortly before hatching, when the cellular plug disappears. Full development and maintenance of the normal cloaca depends on the establishment of a contact between the urodeal sinus and the elements of the Wolffian duct[3]. If neither of the two ducts fuse with the embryonic hind gut, the cloaca undergoes partial involution.

While no parallel information is available in the chicken, it has been suggested by Risley[4] that, in the frog, the perforation of the anus is induced by a secondary order inductor, the mesenchyma. The latter, in turn, receives its inductive powers from the axial structure of the coccygeal region, a primary inductor. This hypothesis received confirmation from the work of Schechtman[5].

By contrast, the earlier investigations of Bytinski-Saltz[6], also carried out on frogs, assign the governing rôle in the induction of anal development to entoderm. Crew[1], attempting to explain in morphogenetic terms the case of a hen with two vents, also suggested that the inductive impulse came from two actively, and independently, growing "tubes", intestine and oviduct. This suggestion seems to have received confirmation in the report of a double-cloaca chicken recorded by Ottoway and Black[2], in which, again, the independent development of the left oviduct led to the formation of a supernumerary cloaca.

In the two examples described in the present report, the entodermal structure (large intestine) was found to be bifurcated a short distance from what normally would have been its terminal point. Each arm of the colon joined a separate cloaca. The two cloacas were equipped with the usual three anatomically recognized regions.

In other words, again an entodermal structure appears to have stimulated the development of a supernumerary cloaca.

It is not likely that the cause-and-effect relationship was the reverse of that suggested. The classical examples of dependence of ectodermal structures, such as eye lens, nasal placodes, and ear vesicles for their genesis and subsequent development on mesodermal and neural tissues point in that direction.

However, this does not explain the underlying cause of bifurcation of the large intestine. The possibility that abortive twinning is involved is an attractive explanation. Byerly and Olsen[7] found that twinning in chickens, though rare, occurs occasionally, with posterior duplications being in preponderance. The appearance of an extra cecum and of a partly duplicated left ureter (first case) together with the bifurcated colon and two cloacas (both cases) suggest such a condition. The phenomenon of twinning, of course, is the expression of divergent and, frequently, independent causative factors[8]. These can be inherent and/or environmental.

The "pear-shaped mass" can be accounted for by assuming that a chaotic condition, perhaps associated with or caused by twinning, existed in the individuation field of the caudal region of the embryo at the time of anal plate and tail differentiation. As Needham[8] points out, the fundamental reason underlying the appearance of such terata is "the failure of the individuation field, at some point early in development, to control the action of evocating substances".

References

[1]Crew, F.A.E.: A Hen with Two Vents. Vet. J., *84*, (1928): 363-365.

[2]Ottaway, C.W., and Black, D.J.C.: Double Cloaca in a Chicken, Vet. J., *100*, (1944): 162-163.

[3]Boyden, E.A.: An Experimental Study of the Development of the Avian Cloaca, with Special Reference to a Mechanical Factor in the Growth of the Allantois. J. Exptl. Zoöl., *15*, (1924): 437-471.

[4]Risley, P.L.: An Experimental Study of Anus Formation in Urodela. J. Exptl. Zoöl., *80*, (1939): 113-129.

[5]Schechtman, A.M.: Experiments on Anus Formation in a Frog Egg. Proc. Soc. Exptl. Biol. and Med., *16*, (1939): 48-50.

[6]Bytinski-Saltz, H.: Untersuchungen über die Induktionfähigkeit der hinteren Medullar-plattenbezirke. Arch. f. Ent.-mech. d. Organ, *125*, (1931): 418-564.

[7]Byerly, T.C., and Olsen, M.W.: Polyembryony in the Domestic Fowl. Science, *79*, (1934): 247-248.

[8]Needham, J.: Biochemistry and Morphogenesis. London, (1942): 228-230.

Recovery of Viable Anthrax Spores from Vapors of Heated Anthrax Spore Suspensions and from the Distillate from Anthrax Spore Suspensions

C. D. STEIN, V.M.D., and HERBERT ROGERS

Washington, D. C.

THE IMPORTANCE of handling cultures, suspensions of microörganisms, spores, and other virulent material to prevent contamination from outside sources, as well as to obviate the possibility of laboratory contamination with the materials being handled, has long been recognized as an essential part of good laboratory technique. More recently, however, in spite of the usually recognized precautions taken to prevent contamination, evidence has come to light that laboratories may become contaminated and laboratory workers sometimes become infected with the microörganisms being handled. Consequently, this question is now receiving more attention. The observations reported in this paper may throw additional light on some aspects of laboratory contamination.

In recent studies on the resistance of anthrax spores to heat, certain observations made by the authors suggested the possibility that viable anthrax spores may escape into the atmosphere during the heating of anthrax spore suspensions in open containers.

PRELIMINARY OBSERVATIONS AND EXPERIMENTS

In an experiment in which anthrax spore suspensions in 2-cc. amounts in pyrex test tubes (15 mm. × 150 mm.) plugged with cotton stoppers were heated for varying periods in an oil bath at 90 to 91 C., with the upper half of tubes protruding above the oil line of the oil bath, it was found that spore suspensions from the same strain that were destroyed by heating for sixty minutes, in some instances, resisted heating for sixty minutes or longer. These irregular results led to observations and tests which showed that the condensation forming on the upper part of the inside of the test tubes just beneath the cotton plugs and above the oil line, as illustrated in figure 1, invariably contained viable anthrax spores. This occurred even after heating for an hour or longer and, in some instances, when the spores contained in the suspension in the bottom of the tubes had been killed by heat.

As a result of these observations, the following preliminary experiment was carried out: Anthrax spore suspensions in sterile distilled water in 2-cc. amounts were carefully transferred to pyrex test tubes of uniform size and thickness (15 mm. × 150 mm.). To obviate the possibility of contaminating the upper portions of tubes

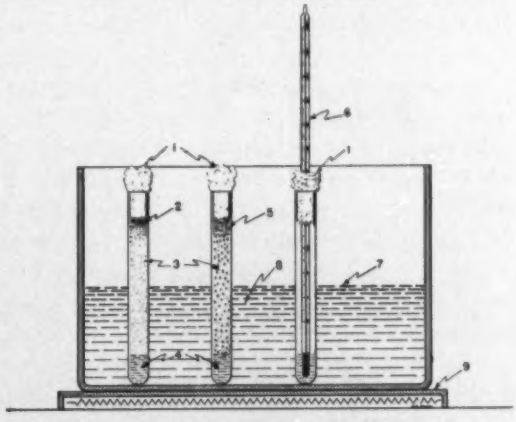


Fig. 1—Method used in heating anthrax spore suspensions in oil bath with automatic temperature control, 90C. to 91C.
(1) Cotton plug; (2) condensed vapor containing living spores; (3) vapor from heated spore suspension; (4) spore suspension; (5) droplets of condensation containing viable spores flow back into spore suspension; (6) thermometer in control tube; (7) height of oil line; (8) hot oil; (9) electric heating device.

From the Pathological Division, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C.

Mr. Rogers was a scientific aide in the experiments.

with anthrax spores, the spore suspensions were transferred to tubes by specially designed, long-stemmed funnels with a sliding glass sleeve. The tubes were plugged with cotton and placed in an oil bath in which a temperature of 90 to 91 C. was automatically maintained. About one-half of the upper portion of the tube protruded above the oil line in the oil bath. After heating for forty to sixty seconds, a very fine condensation formed on the inner and upper part of tubes well above the oil line and just below the cotton plugs and soon developed into small droplets of water. Swab specimens of the moisture, when cultured, were positive for anthrax in all instances. It was further ascertained that, in some instances, droplets of the moisture containing the viable spores flowing down the sides of the tubes reinfected the spore suspension which had previously been destroyed by prolonged heating (*See* fig. 1).

These preliminary observations suggested the possibility that viable anthrax spores may escape into the atmosphere in the vapors arising from spore suspensions during the heating or boiling of such suspensions in open containers. The following experiments were, therefore, carried out to obtain more information on this possibility:

Experiment 1.—Spore suspensions in sterile distilled water from 15 different strains of *Bacillus anthracis* were transferred, in 25-cc. amounts, to 15 sterile pyrex flasks by means of long-stemmed funnels to prevent possibility of contaminating the upper portions of flasks. Flasks were plugged with loosely fabricated cotton plugs and placed on a preheated hot plate. After heating from forty to sixty seconds, the vapors arising from the spore suspension formed a very fine condensation on the neck of the flask below the cotton plugs as illustrated in A, figure 2. Then, the cotton

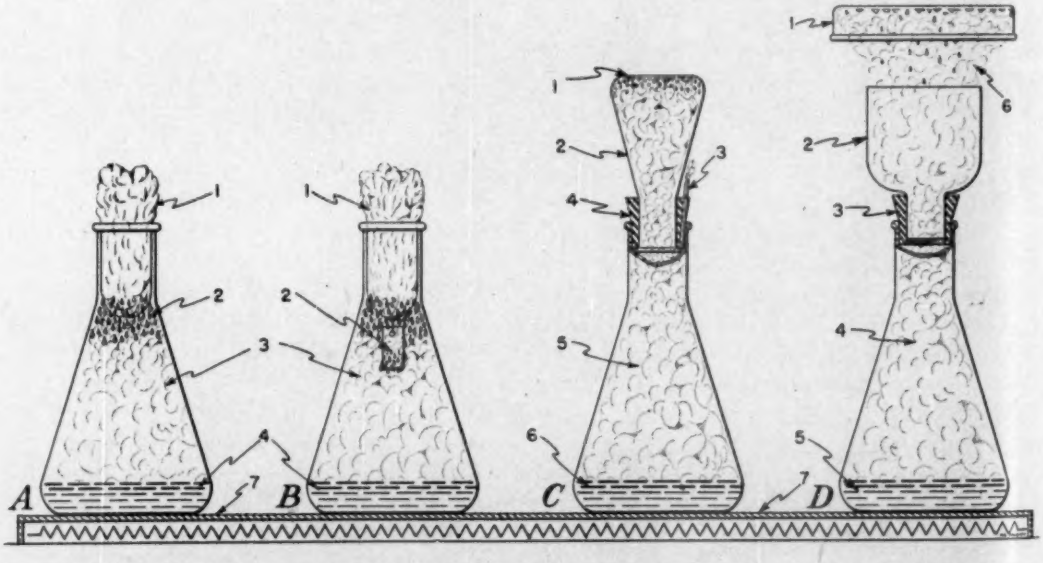


Fig. 2—Methods used to determine whether viable spores escape into the atmosphere during the heating of spore suspensions.

A. (1) Cotton plug; (2) condensation containing viable spores; (3) vapor arising from heated spore suspension; (4) spore suspension.

B. (1) Cotton plug; (2) condensed vapor containing viable spores on metal disc; (3) vapor; (4) spore suspension.

C. (1) Layer of agar on bottom of inverted flask; (2) small inverted flask; (3) air vent; (4) rubber stopper; (5) vapor; (6) spore suspension.

D. (1) Inverted agar plate suspended over funnel; (2) funnel; (3) rubber stopper; (4) vapor from spore suspension; (5) spore suspension; (6) viable spores escape into atmosphere in vapor; (7) heating device.

plugs were quickly removed from each flask and swab specimens were made of the condensation which had formed after heating for forty to sixty seconds. Swabs were placed in tubes containing nutrient broth and incubated. The cotton plugs were quickly replaced, heating continued until boiling began, and boiling was continued for three to five minutes. Swab specimens were then again made from the neck of flasks and incubated. The first group of swabs, made after the suspensions had been heated forty to sixty seconds, were all positive for anthrax, whereas the second group, made after suspensions had been boiled three to five minutes, were all negative. Culture tests made on the spore suspensions in the flasks that had been boiled three to five minutes were also all negative for anthrax.

The results of this test indicated that, during the heating of anthrax spore suspensions, viable spores could invariably be recovered from the condensed vapor found on the necks of flasks before the spore suspension had reached the boiling point. On the other hand, no viable spores could be recovered from the neck of flasks or the spore suspensions themselves after boiling from three to five minutes.

To determine whether viable spores escaped into the atmosphere in the vapors produced when heating spore suspensions of *B. anthracis* and other types of spore-bearing aërobic organisms, the tests described under experiments 2, 3, and 4 were carried out. In these experiments, spore suspensions made from two avirulent strains of *B. anthracis* and one strain of *Bacillus cereus* were used to obviate the possibility of contaminating the laboratory with virulent spores. Spore suspensions in sterile distilled water, prepared from each of the above mentioned three strains, were transferred to sterile pyrex flasks in 25-cc. amounts as previously described under experiment 1.

Experiment 2.—In this experiment, three flasks containing, respectively, avirulent anthrax spore suspension 1, avirulent anthrax spore suspension 2, and a spore suspension of *B. cereus* were plugged with loosely fabricated cotton stoppers. From the center of each cotton plug, a small, sterile metal disc was suspended as illustrated in B, figure 2. The flasks were placed on a preheated hot plate and, after heating for forty to sixty seconds, the cotton plugs were removed and the metal discs were detached from the plugs with a sterile pair of scissors and dropped into tubes of sterile broth and incubated at 37 C. for twenty-four hours. The metal discs from flasks 1 and 2 were positive for *B. anthracis* and the one from flask 3 positive for *B. cereus*. The results of this test indicated that the spore-laden vapor was present in all parts of the upper portions of the flasks after heating from forty to sixty seconds and thus contaminated the cotton plugs. They further suggested that if the flasks were not plugged with cotton stoppers, the spore-containing vapors would pass directly into the atmosphere.

Experiment 3.—In this experiment, as in experiment 2, three flasks containing the three different spore suspensions previously mentioned were plugged with rubber stoppers each having a small vent hole to permit the release of pressure. Into each stopper was inserted a small, inverted flask containing a layer of solidified agar on its bottom surface so as to catch vapors emanating from the large flasks containing spore suspensions as illustrated in C, figure 2. The apparatus was placed on a preheated hot plate and, after heating for forty to sixty seconds, the small inverted flasks were removed, plugged with sterile cotton stoppers, and placed in the incubator. The agar cultures in two of the inverted flasks were positive for *B. anthracis* and those in the other inverted flask were positive for *B. cereus*. The test indicated definitely that the spore-containing vapor had escaped from the large flasks containing the heated spore suspensions into the small, inverted flasks and that the spores were viable.

Experiment 4.—In this experiment, as in experiments 2 and 3, three flasks con-

taining the three different spore suspensions previously mentioned were plugged with rubber stoppers each fitted with an open funnel as illustrated in D, figure 2.

The flasks were placed on a preheated hot plate and, after heating for forty to sixty seconds, a series of plain agar plates with lids removed were inverted and suspended over the top of funnels for short periods as illustrated in D, figure 2. Lids were quickly replaced on the agar plates and they were placed in the incubator at 37 C. for twenty-four hours. A number of the plates suspended over the funnels in the flasks containing the anthrax spore suspension showed typical colonies of *B. anthracis*, whereas those suspended over the funnel in the flask containing suspension of *B. cereus* spores showed typical colonies of *B. cereus*. Swab specimens made from the inside of funnels attached to each of the three flasks were likewise positive after incubation in nutritive broth. The results of this experiment showed, without doubt, that viable spores escaped into the atmosphere in the vapors emanating from spore suspensions heated in open containers.

This experiment was repeated with positive results, using spore suspensions from avirulent strains of *B. anthracis*. Positive results were also obtained, in some instances, by suspending agar plates over the mouth of open flasks after the spore suspensions had been heated for sixty to seventy-five seconds. On the other hand,

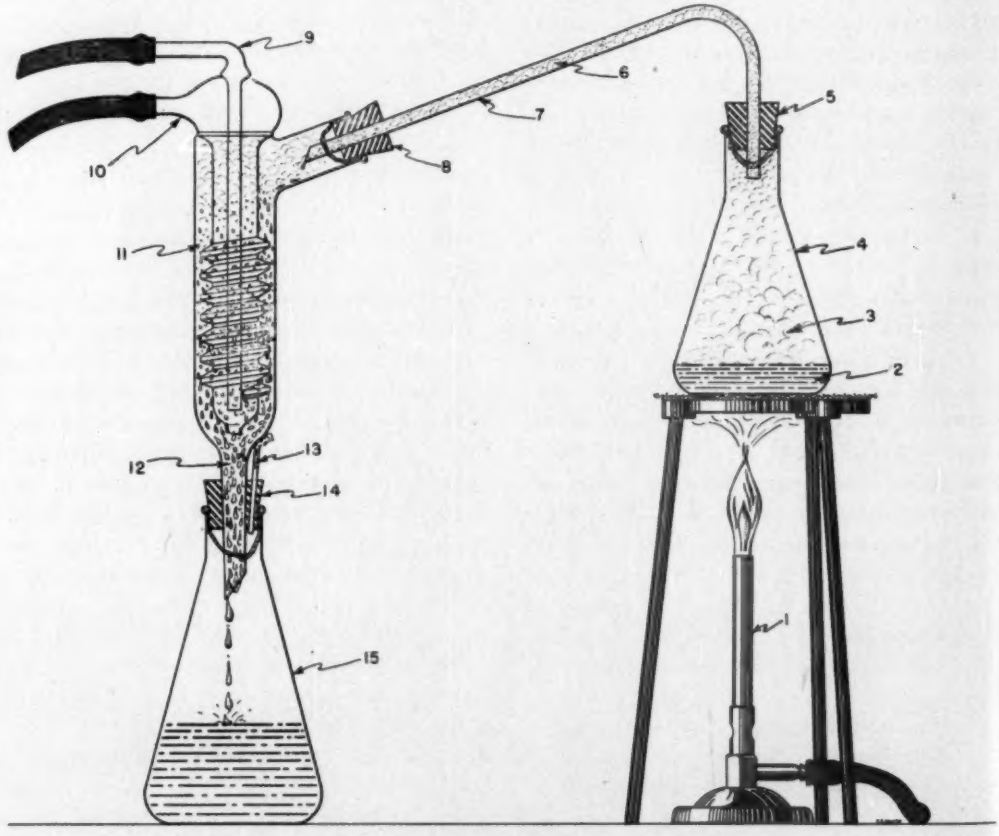


Fig. 3—Apparatus used for distilling anthrax spore suspension.
(1) Bunsen burner; (2) spore suspension; (3) vapor; (4) pyrex flask; (5) rubber stopper; (6) vapor; (7) glass tubing; (8) rubber stopper; (9) cold water inlet; (10) water outlet; (11) Friedrichs condenser; (12) condensed vapor (distillation) containing viable spores; (13) air vent; (14) rubber stopper; (15) flask containing sterile beef-infusion broth.

when spore suspensions had been boiled for three to five minutes, agar plates suspended in the same manner over the mouth of flasks were all negative. These findings indicated that, in the boiling of spore suspensions, the viable spores escaped with the vapors formed during the preliminary heating, but not with the vapors and steam generated after a short period of boiling.

Experiment 5.—Since the previous experiments had demonstrated that during the boiling of anthrax spore suspensions the condensation formed prior to boiling contained viable spores, it was suspected that the distillate from an anthrax spore suspension may likewise contain live spores. To determine this point, the following experiment was carried out: A pyrex flask, into which 50 cc. of an avirulent anthrax spore suspension in sterile distilled water had been carefully transferred as previously described, was connected to a water-cooled Friedrichs condenser by means of rubber stoppers and glass tubing. For receiving the distillation, the condenser, in turn, was connected to a flask containing 300 cc. of sterile nutrient broth by a rubber stopper containing an air vent plugged with cotton (fig. 3). The above apparatus was sterilized in the autoclave previous to being used. The anthrax spore suspension in the flask was slowly heated and brought to the boiling point. Before the spore suspension reached the boiling point, however, condensation of vapor arising from the suspension was noted in the glass tubing leading to the condenser. After the suspension had been allowed to boil for ten minutes, the condenser was detached from the receiving flask containing the sterile nutrient broth, and the flask was plugged with a sterile cotton plug and placed in the incubator at 37 C. After twenty-four hours' incubation, a marked growth appeared in the broth, typical of *B. anthracis*. Seedings were made from the broth culture to agar plates and, after incubation overnight, typical colonies of *B. anthracis* in pure culture occurred on all plates. This experiment indicated that, when distillation is carried out with sterile apparatus, the distillation from an anthrax spore suspension may contain viable spores of *B. anthracis*.

SUMMARY AND CONCLUSIONS

Evidence was obtained that viable spores may escape into the atmosphere when spore suspensions of *Bacillus anthracis* or *Bacillus cereus* in sterile distilled water are heated in open containers and that viable anthrax spores may occur in the distillate from anthrax spore suspensions. Since the vapors arising from spore suspensions during heating may contain viable spores, spore suspensions, especially of *B. anthracis*, should never be heated in open containers.

Distilled water is not necessarily sterile, even when the process of distillation is carried out with sterile equipment, since the condensed vapor from water containing viable spores of an organism such as *B. anthracis* that forms during the preliminary heating may contain some of the living spores.

The Action of Photosensitizing Agents Isolated from Buckwheat

SIMON H. WENDER, Ph.D.
Lexington, Kentucky

FAGOPYRISM, commonly called buckwheat poisoning, is perhaps the most widely known disease produced in domestic animals by light. For more than a century, it has been definitely known to be connected with exposure to sunlight of the sensitized, unpigmented parts of the skin. Hertwig[1], in 1833, reported that the ingestion of the buckwheat plant, *Fagopyrum esculentum*, produced an effect quite similar to that of many narcotics. The surprising features, however, were that only white or white-spotted animals were affected, that the symptoms were noticed after exposure to out-of-door sunshine, and that, if the animals were kept indoors away from the sunlight, they remained normal.

Throughout the nineteenth and the beginning of the twentieth centuries, scientists, mainly veterinarians, continued to report numerous cases involving their observations of animals afflicted with fagopyrism. Although the symptoms varied with the animals and with the intensity and duration of sunlight, the symptoms generally reported consisted of severe itching, pruritus, erythema, edematous swellings of the face and ears and, in many cases, convulsions, cerebral excitement, paralysis, and even death. Among the field animals reported to have become light-sensitive after the eating of buckwheat were cows, sheep, and goats. Reviews on these earlier reports are to be found in the articles by Merian[2], Lutz and Schmid[3], and Mathews[4].

This paper is a contribution from the laboratories of the Division of Agricultural Biochemistry of the University of Minnesota and the C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis and contains material from a dissertation presented by Simon H. Wender to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the Ph. D. degree, June, 1938.

The author is now an associate professor in the Department of Chemistry, University of Oklahoma, Norman, Okla.

The author acknowledges the advice and inspiration given him by his supervisors in this research, the late Dr. Ross Aiken Gortner, university advisor, and the late Dr. Ondess L. Inman, director of the Kettering Foundation. The author also thanks Dr. Paul Rothemund of the Kettering Foundation, for his experimental suggestions during the progress of this research work. The work was facilitated by a generous grant from the Mrs. Mary W. Harriman Trust Fund.

Some Previous Physiological Studies

Oehmke[5], in 1908, fed buckwheat to white mice, guinea pigs, and rabbits and, on exposure to direct sunlight, observed manifestations similar to the fagopyrism observed in farm animals. After long exclusive feeding with Fagopyrum, the animals died on long exposure to diffuse daylight. Their hair could be pulled out in large bunches. An autopsy revealed swelling of the heart, lung edema, and early stages of peritonitis. The animals kept in darkness were unharmed.

Sheard, Caylor, and Schlotthauer[6] sensitized white guinea pigs, swine, and goats with freshly cut buckwheat. Microscopic examination of the sensitized animals with fatal reaction showed the lack of marked pathologic change. Lesions such as petechial hemorrhage of the lungs, brain, liver, stomach, and kidneys suggested that death was due to profound toxemia which killed the animal before extensive anatomical changes developed. In animals experiencing severe reaction, there was marked increase in the coagulability of the blood. It is difficult to draw blood from such animals.

Other Photosensitizing Plants

Meanwhile, other photosensitizing plants were recognized and studied, some for more than a century. One of the most clear-cut photosensitivities is the disease of domestic animals known as hypericism, a disease long recognized as being due to the grazing of domestic animals on the common weed, St. Johnswort (*Hypericum perforatum*), with subsequent exposure to sunlight. A serious disease of sheep occurring in South Africa and known as *geeldikkop*

has been shown by Rimington and Quin[7] to result from abnormal sensitivity to light, although differing in certain important respects from fagopyrism and hypericism. Clare[8] has made some intensive studies on photosensitivity in New Zealand. Mathews[9] found that a part of the symptoms which follow feeding on *Agave lechuguilla*, found in the arid regions of New Mexico, Texas, and Mexico, is a result of photosensitization. Other plants suspected of possibly producing photosensitization include lucerne or alfalfa (*Medicago sativa*), burr clover or trefoil (*Medicago denticulata*), some true clovers (*Trifolium* sp.), vetch (*Vicia* sp.), *Tetradymia glabrata* (producing Bighead), and others. As very little experimental evidence has been reported in these latter cases, it is not yet possible definitely to know their etiology. Blum[10] gives an excellent critical review of the diseases produced in animals by light.

ETIOLOGY OF FAGOPYRISM

At first, it was thought that the etiology of fagopyrism was to be found in insects or in lower organisms which were believed to live on the plants as parasites, and that these suffered certain changes under the influence of sunlight. It later became evident, however, that the plants contained certain chemicals capable of producing a photosensitizing action. The etiology of hypericism has now been learned, and strongly suggests a similarity to fagopyrism. Pace and MacKinney[11] have isolated a red fluorescent pigment from St. Johnswort. Pace[12] has demonstrated that administration of the pigment, hypericin, by mouth, causes typical photosensitization in white rats, and has proved that hypericin is the photosensitizer responsible for the production of the syndrome of hypericism in domestic animals.

Yet, in spite of the many productive experiments on fagopyrism, prior to this study, no one had been able to isolate from buckwheat any compound or compounds which, on feeding to white animals, would cause photosensitization. Some progress in this direction has been made by Oehmke[5], J. Fischer[13], Lutz and Schmid[3], and Chick and Ellinger[14]. Recently, however, Wender, Gortner, and Inman[15] finally succeeded in adapting for use an experimental procedure for the isolation and crystallization of three photosensitizing substances from buckwheat. Each of these three substances (called A, B, and C) isolated from the buckwheat proved to be a photosensitizing agent when fed to white guinea pigs. The chemical details of the isolation and some chemical properties of the isolated substances have been described in another journal[15]. In that work, the buckwheat, *F. esculentum*, was cut at the stem just above ground, when flowering. The entire upper plant was then air-dried and ground. The dried buckwheat was still capable of photosensitizing white guinea pigs when tested seven months after its storage. The methods of isolation of substances A, B, and C from the air-dried buckwheat plant involve extraction with proper solvents, chromatographic adsorption analysis, partition between immiscible solvents, and crystallization.

Substance A is purplish black; insoluble in methyl alcohol, ethyl alcohol, or benzene, but soluble in hot, glacial acetic acid. Hydrochloric acid, at 16 per cent or higher extracted A from ether. The melting point is above 375 C. The two major bands in absorption spectrum of A in glacial acetic acid are at 592.1 and 548.4 mμ.

Substance B is somewhat soluble in methyl alcohol or benzene, and quite soluble in hot acetone and glacial acetic acid. Its solutions are red. Hydrochloric acid, at 23 per cent or higher, extracted B from ether. Its melting point is above 375 C. Two major bands in the absorption spectrum of B in glacial acetic acid are at 589.4 and 545.6 mμ.

Substance C is slightly soluble in methyl alcohol and ethyl alcohol and very soluble in acetone, in ether, and in glacial acetic acid. It could be removed from ether with 5 per cent sodium carbonate solution. The two major bands in the absorption spectrum of C in neutral ether are at 584.7 and 542.7 mμ.

Each of the three substances yielded flu-

orescent solutions. Not only are the absorption spectrums obtained for these substances, A, B, and C, similar to each other, but they are surprisingly similar to the absorption spectrums of hypericin as found by Pace and MacKinney[11]. These authors suggested that this photodynamic substance is a partially reduced polyhydroxy derivative of helianthrone.

The present article describes the animal experiments with the isolated substances, A, B, and C.

ANIMAL EXPERIMENTS

White guinea pigs were used in the experiments described. Each of the animals was first closely observed for at least ten days prior to the experiments for any abnormalities in behavior. None of the animals used had been previously photosensitized or fed any buckwheat. The three isolated substances, A, B, and C, were each fed separately to different animals in doses of 2 to 5 mg. each, inside a gelatin capsule (Eli Lilly No. 5). The gelatin capsule was hand fed to the animal to insure ingestion.

Each day a white guinea pig was fed a capsule containing substance A; another white guinea pig was fed a capsule of substance B; and a third white guinea pig was fed a capsule of substance C. In addition, each of these animals—and a fourth white guinea pig used as a control—were fed on a diet of lettuce, cabbage, carrots, and oats.

Experimental Results.—After forty-five minutes' exposure to the afternoon sunlight on the second day of feeding, the experimental animals showed extreme reddening of the ears, but no other significant changes. On the sixth day of feeding, they were exposed for two and a half hours in very weak sunlight. The animal to which substance B had been fed whined incessantly after about thirty minutes in the light and moved about nervously, twitching, gulping, and at times scratching vigorously. Every now and then, it appeared to become semiparalyzed. Although apparently photosensitized, more symptoms were awaited before one could pronounce the animal definitely sensitized. The animal fed on C was quite nervous and excited. Its ears had begun to flop over and hang loosely, and they were blood red. The guinea pig fed on A remained, most of the time, with its head buried in a dark corner. The latter 2, although definitely different from the control in sunlight, could not be assuredly assumed at the time to be photosensitized.

The next day the animal to which B had been fed, and which had given so many signs of photosensitization the day before, was found dead. An autopsy revealed a clotting of the blood throughout the body, and inflammation and swelling of the intestines. Another white guinea pig was now selected to be used as the test animal for substance B.

On the fourteenth day of feeding of substances A and C (sixth day of substance B), the sun was shining brightly. Since the weather was still too cold, the animals were not taken out of doors, but instead were moved into a nearby greenhouse to test for photosensitization. Within ten minutes after exposure to the sunlight coming through the glass of the greenhouse, the animals fed on A and C were whining and were very irritable and somewhat excited. After forty-five minutes in the light, every one of the animals, except the control, exhibited definite proof of being photosensitized. On this occasion, the animal fed C was most highly sensitized. The guinea pig was running back and forth violently, nervously scratching its face and nose, rubbing its sides and hind legs, vigorously gnawing and biting the cage wire, whining pitifully, and trying to hide in corners. The snout was badly swollen, the mucous lining of the nose appeared quite distended, and the ears were extremely red. The animal would rub energetically against the food containers and projecting parts of the cage, and had to be removed to the dark to be calmed.

After exposure for slightly more than an hour, the other 2 experimental animals, but not the control, were exhibiting such violent spasms, irritations, shiverings, and scratchings that they had to be taken into

the dark also. Although the symptoms of these other 2 animals were somewhat the same as those of the animal fed C, they were not quite as violent. Moreover, the snouts did not appear swollen, although they were red. Slightly different symptoms were noticed, however. The animal to which B had been fed, after some of its energetic motions, would fall over on one side and lie there for minutes, apparently paralyzed in the hind quarters, while gasping for breath. The animal could be touched and pushed, but was unable to move. The animal fed A would dive into corners with its face away from the sun, and at times would slide along on its stomach, and stretch out its neck and rub it on the food container. However, each of the animals behaved normally the next morning, when kept in the dark.

A study of the animals the day after the exposure mentioned revealed that the animal fed A had become quite pigmented. The ears and snout, formerly very pink and light, and even the bottom of the legs, had turned black. The inside of the mouth and the tongue appeared normal. The ears of the animal fed C also were dark and hanging very loosely.

Twice more within a week, the animals were exposed to very weak sunlight inside the greenhouse. Again definite signs of photosensitization were exhibited. The violent motions of the fourteenth day, however, were not attained. The feeding of the substances isolated from the buckwheat was discontinued on the seventeenth day after the beginning of the feeding experiments. However, to see if the animals were still photosensitized, on the thirtieth day after the feeding experiments had started, the animals were taken out of doors into bright sunlight. The animal fed B, after moving about nervously, would apparently get strokes of paralysis, falling over on its side or on its stomach unable to move, even on being pushed or handled. At times, a spasm or twitching would occur and the animal would unsuccessfully try to rub its snout while lying on its side or stomach with legs stiffly spread out. Soon it had to be taken out of the light. After an hour in the dark, it behaved normally again. The animal fed C also exhibited its usual photosensitized actions, but not quite as violently as on the fourteenth day. Definite symptoms were not obtained at this exposure with the animal fed A; it was now very highly pigmented.

Although the symptoms of the guinea pigs fed on the substances isolated from buckwheat appeared, on occasions, not to be as violent nor as prolonged as those shown, at times, by animals sensitized with the buckwheat plant itself, the symptoms were clear-cut and unmistakable. One may conclude, therefore, that each of the three substances, A, B, and C, isolated from buckwheat, is a photosensitizing agent when fed to white guinea pigs.

SUMMARY

Three substances have been isolated and crystallized from the buckwheat plant. Each of these three substances has been found to produce photosensitization when fed to white guinea pigs. These animal experiments have been described.

References

[1]Hertwig: Praktische Arzneimittellehre für Tierärzte. Berlin, 1833.
[2]Merian, L.: Archiv für Anatomie und Physiologie, Abt. I, (1915): 161-188.
[3]Lutz, H. E. W., and Schmid, G.: Fagopyrism—Biochemical Investigation and Critical Study of its Pathogenesis. Biochem. Ztschr. *266*, (1930): 67.
[4]Mathews, F. P.: Photosensitization and the Photodynamic Diseases of Man and the Lower Animals. Arch. Path. *23*, (1937): 399-429.
[5]Oehmke, W.: Sensitiveness of White Animals to Light after Consumption of Buckwheat. Zentralbl. f. Physiol., *22*, (1908): 685.
[6]Sheard, C., Caylor, H., and Schlotthauer, C.: Photosensitization of Animals After the Ingestion of Buckwheat. J. Exptl. Med., *47*, (1928): 1013.
[7]Rimington C. and Quin, J. I.: The Nature of the Photosensitizing Agent in Geeldikkop. Onderstepoort J. Vet. Sci. Anim. Indust., *3*, (1934): 137.
[8]Clare, N. T.: Photosensitivity Diseases in New Zealand, Part IV. New Zealand J. Sci. and Tech. *27*, (1945): 23.
[9]Mathews, F. P.: Lechuguilla Poisoning. Texas Agric. Exper. Sta. Bull, 554, 1937.
[10]Blum, Harold F.: Photodynamic Action and Diseases Caused by Light. Reinhold Pub. Corp., New York, N. Y., 1941.
[11]Pace, N., and MacKinney, G.: Hypericin, the Photodynamic Pigment from St. Johnswort. J. Am. Chem. Soc., *63*, (1941): 2570.
[12]Pace, N.: The Etiology of Hypericism, a Photosensitivity Produced by St. Johnswort. Am. J. Physiol., *136*, (1942): 650.
[13]Fischer, J.: Inaug. Dissert. Berlin, Köln-Ehrenfeld (1909).
[14]Chick, H., and Ellinger, P.: The Photosensitizing Action of Buckwheat. J. Physiol., *100*, (1941): 212.
[15]Wender, S. H., Gortner, R. A., and Inman, O. L.: The Isolation of Photosensitizing Agents from Buckwheat. J. Am. Chem. Soc., *65*, (1943): 1733.

Reproductive Failure and Agalactia in Ergotized Female Rats

A. W. NORDSKOG, Ph. D.

Bozeman, Montana

A RECENT PUBLICATION (Nordskog and Clark[1]) has shown that experimentally produced ergotism in brood sows resulted in total loss of the baby pigs. In this work, 10 sows fed barley ergot in concentrations from 0.1 to 1.0 per cent during pregnancy farrowed small and weak pigs, about half of which were dead at birth. Almost complete lack of udder development, with no evidence of milk secretion, was observed. All pigs born alive died within one or two days, from starvation. In the same paper, an experiment was reported on feeding barley ergot to bred female rats. Nine females fed rations containing 1.0, 0.5, or 0.05 per cent ergot failed to produce any young. Two animals, put on 3.0 per cent and 1.0 per cent barley ergot, respectively, about twelve days after breeding, each produced litters, but all individuals died within a few days, apparently due to starvation. The writer is not aware of any other published reports relating to agalactia produced experimentally with ergot.

Most studies on ergotism have been concerned with ergot from rye. The above study was carried out with barley ergot. Although the same species of organism (*Claviceps purpurea*) may infect any of the small grains as well as several different grasses, it would be informative to compare the effects of ergot from different sources (grains) on reproduction and lactation. This report deals with experiments using ergot from rye, barley, wheat, and grass (western wheat) fed to young, but sexually mature, male and female rats. In addition, a separate experiment is reported employing one of the principal alkaloids from ergot—ergotoxine. Rats were used as experimental animals since only small quantities of the different ergots and ergotoxine were available.

Contribution from Montana State College, Agricultural Experiment Station. Paper No. 88 Journal Series.

The author is now on the staff of the Department of Poultry Husbandry, Iowa State College, Ames, Iowa.

The writer is indebted to the Home Economics Research Department of the Montana Agricultural Experiment Station for the laboratory space for this work. Special thanks are due Mrs. Helen Mayfield and Mrs. Marjorie Hedrick of the same department for valuable assistance in the laboratory and to Dr. C. A. Tryon, of the Department of Zoölogy and Entomology, for his generous assistance in obtaining data on vaginal smears.

EXPERIMENTAL

In experiment 1, 25 bred female rats were divided into five lots of 5 animals per group. Before breeding, these were young virgin animals, obtained commercially (Sprague-Dawley, Madison, Wis.). Within each of the first four lots, five different samples of ergot were fed. The first was obtained from grass hay (western wheat); the second and third samples from wheat and rye, respectively; and the fourth and fifth from barley of two different years, 1942 and 1944, respectively. The 1942 barley ergot was the same as used in the study previously reported in swine and rats.[1] After mating the animals, vaginal smears were taken daily to determine the date of breeding. Females were put on experiment not later than two days after sperm cells were noted in the smears. The bred females were weighed and put in separate cages. Weights were then taken twice weekly (alternately the third and fourth days). Food consumption was measured to correspond with the weighing periods.

Each treatment ration contained 1.0 per cent ergot. The basal ration was a commercial stock feed (Purina Dog Chow). The ergot samples and the basal feed were ground to the same degree of fineness. Lots 1 and 2 were fed the ergot rations throughout the experiment. Lot 3 was fed the ergot rations up to the tenth day after breeding followed by the ergot-free ration during the remainder of the experiment. Lot 4 was fed ergot-free ration through the tenth day after breeding followed by the ergot rations for the balance of the trial. Lot 5, which served as controls, received the ergot-free ration throughout.

In experiment 2, the alkaloid, ergotoxine,

was used as the test material on two series of rats. Series A consisted of bred females and series B of lactating females. Twelve bred female rats in series A were lotted into three groups of 4 each. Lot 1 received a ration containing ergotoxine base (30 mg. per kg. of basal feed). Lot 2 was fed the same except that ergotoxine ethansulfonate was used. Lot 3 represented control animals on the basal diet (Purina Dog Chow). In lots 1 and 2, the animals were started on the treatment rations at varying lengths of time after breeding from the second day to the thirteenth day.

In series B, females in different stages of lactation were fed ergotoxine base at the same rate as above in order to determine the effect on growth reponse and survival of the young.

In addition to these major experiments, minor ones were conducted to obtain information on: (1) the effect of ergot on fertility in males; (2) the effect of ergot on

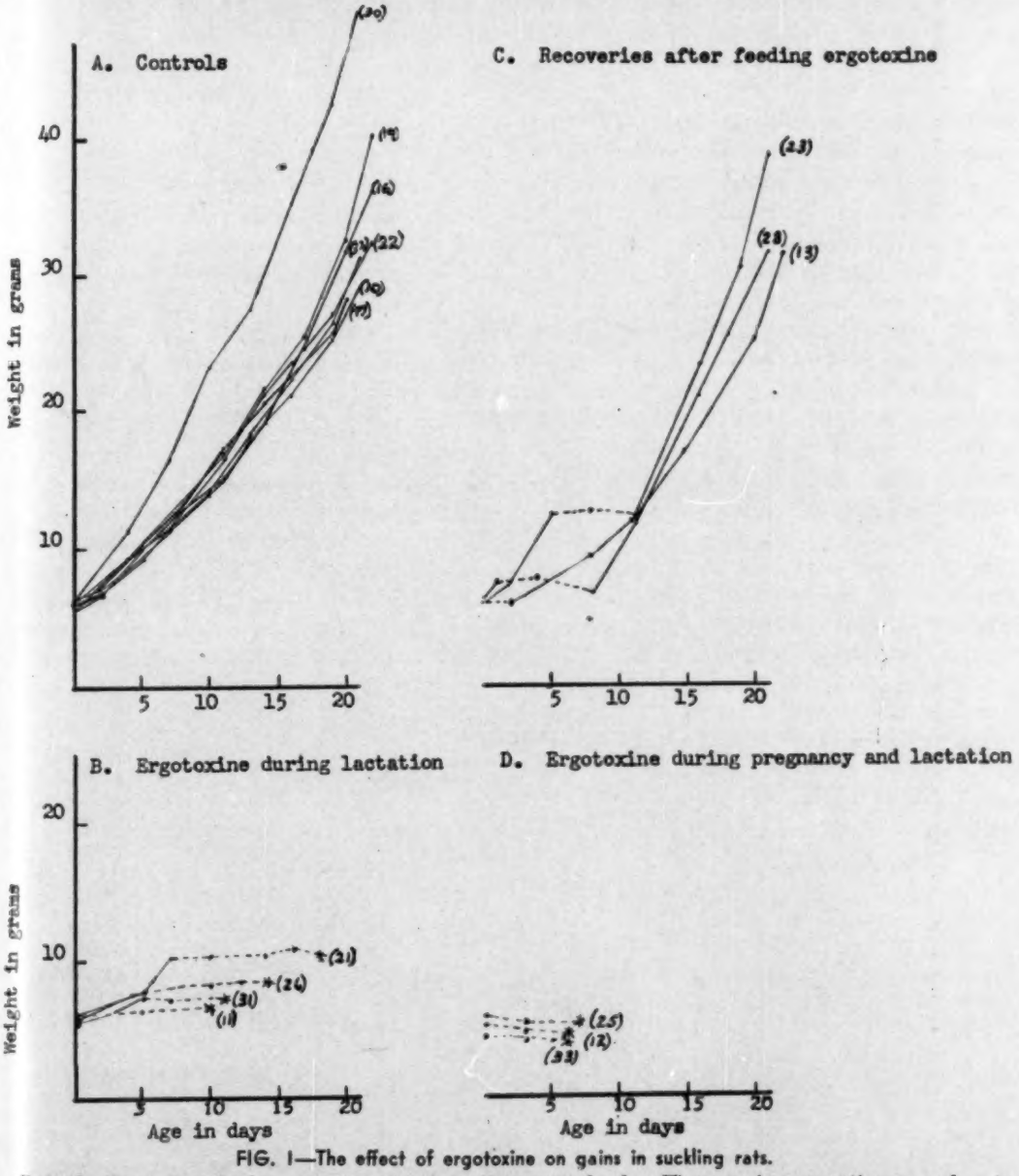


FIG. 1—The effect of ergotoxine on gains in suckling rats.

Dotted line = ergotoxine treatment. * = all young dead. Figures in parentheses refer to dam's number.

TABLE I—The Effect of Ergot from Different Sources on Reproductive Performance and Survival of Young of Female White Rats

Lot	Treatment: Period of gestation on ergot	Treatment: Kind of ergot fed	Rat No.	Gain: Initial wt. (Gm.)	Gain: Period A[a] (Gm.)	Gain: Period B[a] (Gm.)	Av. daily feed consumption: Period A[a] (Gm.)	Av. daily feed consumption: Period B[a] (Gm.)	Total amount ergot consumed: Period A[a] (Gm.)	Total amount ergot consumed: Period B[a] (Gm.)	Number of young born: alive	Number of young born: dead	Av. birth wt. live young (Gm.)	Remarks
1	Entire	Grass	17	170	14	5	8.9	12.1	.71	1.33	....	....	...	
		Wheat	18	188	8	6	9.0	11.5	.72	1.27	....	....	...	
		Rye	29	200	5	7	10.4	12.5	.83	1.42	....	....	...	None in lot 1 produced any young.
		Barley (1942)	21	172	20	13	10.6	13.1	.85	1.44	....	....	...	
		Barley (1944)	14	202	− 2	− 4	9.1	11.4	.73	1.26	....	....	...	
		Average		186	9.0	5.4	9.60	12.12	.768	1.344	None			
2	Entire	Grass	10	254	−18	−10	11.0	12.1	1.10	1.21	....	....	...	
		Wheat	30	178	18	2	11.3	11.8	.90	1.30	....	....	...	
		Rye	25	195	− 5	8	10.4	11.6	1.04	1.16	....	....	...	None in lot 2 produced any young.
		Barley (1942)	19	219	11	5	11.7	12.0	1.05	1.20	....	....	...	
		Barley (1944)	32	200	− 4	11	11.2	12.0	1.12	1.20	....	....	...	
		Average		209	0.4	3.2	11.12	11.90	1.024	1.214	None			
3	A[a]	Grass	33	171	14	17	10.7	13.2	.96	None	....	....	...	
		Wheat	11	215	−16	25	11.2	15.7	1.01	None	....	....	...	
		Rye	16	228	− 7	2	10.1	14.1	.81	None	....	....	...	None in lot 3 produced any young.
		Barley (1942)	28	212	−18	31	11.0	15.2	.99	None	....	....	...	
		Barley (1944)	23	199	− 1	15	11.4	14.1	.91	None	....	....	...	
		Average		204	− 5.6	18.0	10.88	14.46	.936	....	None			
4	B[ab]	Grass	15	227	7	41	14.0	12.6	None	1.51	6	None	5.7	All young dead by third day.
		Wheat	22	210	10	51	12.7	14.0	None	1.54	10	None	4.9	All young dead by seventh day.
		Rye	31	201	− 7	83	14.8	13.2	None	1.58	10	None	4.8	All young dead by fourth day.
		Barley (1942)	12	200	24	47	13.7	11.6	None	1.16	9	None	4.9	All young dead by seventh day.
		Barley (1944)	26	217	16	78	15.9	16.4	None	1.81	10	None	4.9	All young dead by third day.
		Average		211	10.0	60.0	14.22	13.56	....	1.520	9.0	None	5.04	
5	No ergot fed (controls)	....	34	210	24	74	15.6	16.1	None	None	10	None	5.8	9 young raised to weaning.
		....	24	206	16	100	16.2	20.9	None	None	10	None	5.9	All young dead within 9 days.
		....	27	211	23	73	15.9	15.2	None	None	8	2	6.8	All young eaten by mother.
		....	20	254	24	60	16.4	18.1	None	None	7	None	6.3	6 young raised to weaning.
		....	13	245	17	67	....	....	None	None	8	None	5.6	Only 1 young raised to weaning.
		Average		225	20.8	74.8	16.03	17.58	....	....	8.6	.4	6.08	

[a]Period A=First to tenth day post-breeding; Period B=Tenth to twenty-first day post-breeding.

[b]Lot 4 females continued on ergot after littering.

fertility and the estrous cycle in females. (3) the effect of ergot and ergotoxine on palatability of a ration.

All animals in both experiments were weighed periodically and feed consumption was recorded. The rations were fed *ad libitum*. All experimental rations contained 1.0 per cent ergot or 3 mg. per cent ergotoxine.

RESULTS

Experiment 1. *A Comparison of Ergot Samples from Different Sources on Reproduction and Survival of Young.*—Data obtained from this experiment are given in table 1. The results indicate that each of the sources of ergot yields almost exactly the same effect. It is of interest to note that the 1942 barley ergot was still potent. This sample had been finely ground and stored in an ice box for over one year.

No young were produced in either of the first three lots. These lots were fed the ergot rations either for the entire experimental period or to the tenth day after breeding (lot 3) followed by the control feed. Lot 4 was started on the ergot rations on the tenth day post-breeding. All females in the lot produced young. They were continued on these rations after littering. Birth weights of the young averaged over 1 Gm. less than those in the control group (lot 5). This difference is highly significant statistically ($P < .01$). All young in lot 4 failed to gain in weight and died within a few days. Results were identical to those already reported[1]. Milk in the stomachs was observed soon after birth but, on the second or third days, the young were no longer receiving any nourishment.

Some doubt as to whether ergot was solely responsible for these losses is suggested by the results from the control group. Only three litters in lot 5 were raised to weaning and rat 13 lost all but 1. However, rat 27 failed to mother her young after littering (they were eaten soon after birth) and rat 24 mothered hers very poorly. Perhaps this might be explained partially by the fact that all females were with their first litters.

Average daily feed consumption tended to be greater in period B (second ten days after breeding) than in period A (first ten days after breeding) except for lot 4. Those animals on the ergot rations during period A (lots 1, 2, and 3) consumed an average of 4.5 Gm. less than those on the basal ration (lots 4 and 5). This difference is statistically significant and probably can be explained by a difference in appetite between these groups as well as by a difference in palatability. Distaste for the ergot rations is indicated by the difference in average daily feed consumption by periods in lots 4 and 5. The females in lot 3

TABLE 2—The Effect of Ergotoxine on Reproductive Performance and Survival of Young in Female White Rats

Lot	Treatment	Rat No.	Days after breeding on treatment	Daily feed consumption to 21st day post-breeding (Gm.)	Total amt. ergotoxine consumed (mg.)	Number of young born Alive, Dead (A. D.)	Av. birth wt. live young (Gm.)	Remarks
1	Ergotoxine	24	2nd-21st	14.4	8.19	None	...	No young produced.
		18	5th-21st	11.9	6.81	None	...	No young produced.
		12	10th-27th	14.2	4.68	10 0	5.3	All young dead on sixth day.
		25	13th-28th	12.7	3.06	8 0	6.0	All young dead on seventh day.
2	Ergotoxine ethansulfonate	29	2nd-21st	11.8	6.72	None	...	No young produced.
		15	3rd-19th	12.1	5.79	None	...	No young produced.
		33	5th-26th	12.3	6.27	7 1	4.6	All young dead on fifth day.
		27	11th-12st	11.6	4.86	None	...	No young produced.
3	Control ration	14	Entire	14.6	None	None	...	Failed to become pregnant.
		16	Entire	14.8	None	9 1	5.5	8 normal young at weaning.
		17	Entire	16.5	None	10 0	5.5	10 normal young at weaning.
		32	Entire	13.1	None	11 0	5.7	11 normal young at weaning.

averaged 3.5 Gm. of feed more per day per animal after the basal ration was substituted for the ergot. This was not influenced by pregnancy since these animals failed to litter. In lot 4, daily feed consumption was less on the ergot ration, in spite of the fact that their requirements should have been higher during the latter stages of pregnancy, and which is indicated by the difference in feed consumption between periods in lot 5.

Gains shown by periods in table 1 mainly are reflective of whether or not females are with young. Very large gains are noted only in period B of lots 4 and 5. The females in these two lots all produced litters twenty-two days after breeding. The differences in gains in period B between these two lots are probably real, but the numbers are too small to show statistical significance.

Summarizing experiment 1, differences between sources of ergot on reproductive performance in female rats could not be demonstrated. Therefore, the same toxic principle must be contained in each of them. Ergot fed at a 1.0 per cent level *ad libitum* during the first ten days after breeding probably causes very early resorption of the embryos or fetuses. Ergot fed at the same level starting at ten days of pregnancy does not prevent the females from carrying their young to term. However, the young tend to be below average weight at birth. If females with litters are continued on an ergot diet after littering, the young appear to die from starvation.

A. Control

B. Ergotoxine during lactation

C. Ergotoxine during pregnancy and lactation

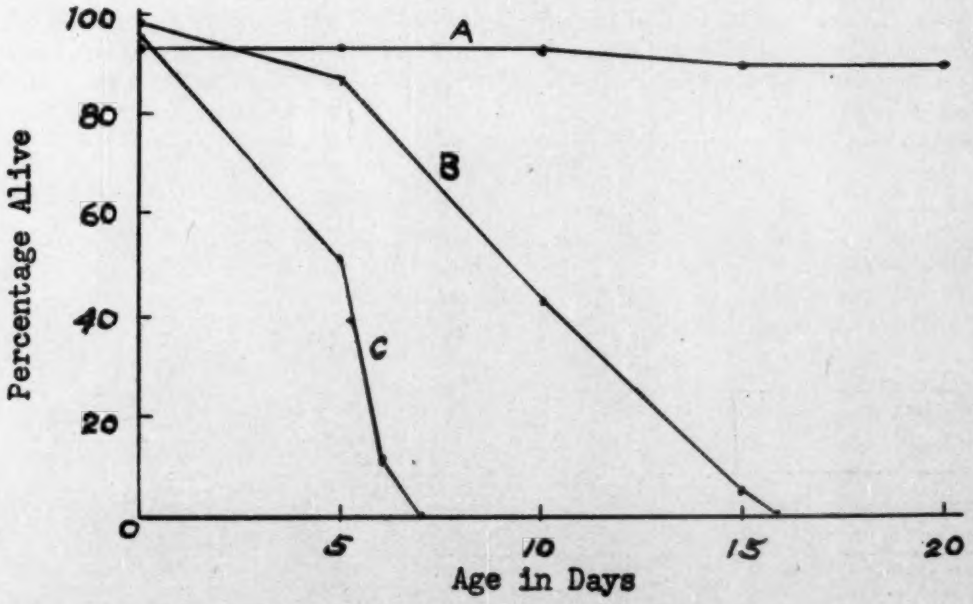


FIG. 2—The effect of ergotoxine on mortality of suckling rats.

Experiment 2. *The Effect of Ergotoxine on Reproduction and Survival of Young.*— It would be of value to know the specific toxic principle found in ergot which prevents normal reproduction and lactation in rats. Since the alkaloids of ergot have long been known to have specific toxic properties, it is logical that these should be studied relative to their possible effects on reproduction and lactation. Two sources of ergotoxine were available for this work. One was a very pure sample of ergotoxine base* and the other was its salt (ergotoxine ethansulfonate). The alkaloids were dissolved in hot alcohol and mixed with finely ground stock feed so that 1 kg. of feed contained 30 mg. of ergotoxine. Although no tests of ergotoxine content were made on whole ergot, this amount would probably be equivalent to about 3 per cent of whole ergot. (*See* Fitzhugh *et al.*[2]).

Twelve females used in the first experiment were rebred for series A in experiment 2. The results are reported in table 2. Two females did not produce litters in lot 1 on the ergotoxine base. These were started on experiment on the second and fifth days post-breeding. Two females produced young which were put on the ergotoxine ration on the tenth and thirteenth days, respectively. These females were continued on treatment after littering. All of the young were dead within a week after birth. Only one litter was produced (female 33) in lot 2 on ergotoxine ethansulfonate. The rats in this litter were very small at birth and none lived more than five days. The dam was on the ergotoxine ration from the fifth day after breeding. It is not clear why rat 27, on the treatment diet beginning with the eleventh day after breeding, failed to produce a litter. In the control group (lot 3), three litters were produced and 1 female failed to become pregnant. All the control litters grew normally to weaning age. The average daily feed consumption for rats on treatment was less than for those on the control diet.

In order to determine whether ergotoxine is able to inhibit milk secretion in otherwise normal lactating animals, ergotoxine (at the same level as fed previously) was added to the rations of 7 females with normal litters in series B. Observation of the young indicated that a marked reduction of milk consumption occurred within short time†. The young failed to gain weight, showing evidence of starvation. Four litters were lost. The remaining three, at the point of death, made rapid recoveries upon removal of the ergotoxine. These results are shown graphically in figure 1. Growth curves of litter averages (individual rat basis) are given for 7 controls in figure 1-A, four litters on ergotoxine in figure 1-B, three litters showing recovery after feeding ergotoxine in figure 1-C, and three litters whose dams were on ergotoxine during the latter phase of pregnancy and also after littering, in figure 1-D (data on these are shown also in table 2). The depression on litter gains in weight, from feeding ergotoxine to the dams, is clearly shown from these growth curves.

The effect of ergotoxine on mortality of young is shown in figure 2. The young from females receiving the alkaloid during both pregnancy and lactation show a more rapid rate of mortality than those on treatment during lactation only.

OTHER OBSERVATIONS

Several additional observations were made. The question arose as to whether ergot might have an effect on the estrous cycle and fertility of the female rat. Four females (10, 19, 22, and 30) which were on ergot treatment in experiment 1 were continued on ergot. Only 1 of these (22) littered in the first experiment. Daily examination of the vaginal smears indicated only normal changes in vaginal epithelium and period of estrus. These females were

*This was kindly donated by the Treemond Sales Company, Brooklyn, N. Y.

†In suckling rats, before the coat of hair has developed, the presence of milk in the stomachs can be observed through the skin as whitish masses.

then bred, the ergot feeding discontinued, and normal litters were produced.‡

To obtain information on the effect of ergot on the breeding ability of males, 3 animals were fed *ad libitum* on a 1.0 per cent ergot ration. After one month on the treatment each was mated to females (13, 26, and 31)§ all of which became pregnant. A second test was made after two months' treatment. Again each male proved to be fertile. During this time, the males on treatment showed normal gains in weight as compared with controls and exhibited no external signs of ergotism.

Ergot in rations is known to be distasteful to animals. Ergotoxine base at the rate of 30 mg. per kilogram of feed is apparently unpalatable as well. Three groups of females (4 in each of two, 3 in the other) were fed free-choice, in separate containers, either ergotoxine or ergot rations and, separately, basal feed. Lots 1 and 2 were each fed the ergotoxine ration and the basal feed. Lot 3 was given an ergot ration plus basal feed. Daily consumption of experimental and control rations was measured for six consecutive days. Of the total feed consumed in lot 1, 28 per cent was of the ergotoxine ration. Lot 2 ate 34 per cent of the ergotoxine ration, and lot 3 consumed only 17 per cent. It is evident that the 1 per cent ergot ration was very unpalatable. Even the small quantity of 3 mg. per cent of ergotoxine made the feed unpalatable.

DISCUSSION

Beginning with the eighth day after breeding, each female was examined for the "placental sign" of pregnancy, a bloody vaginal discharge. In normal female rats, this usually reaches its maximum on the fifteenth day of pregnancy. No placental signs were observed in any females of experiments 1 and 2 which failed to litter. All females producing litters appeared to be normal with respect to placental sign. Female rats on vitamin E-free diets are known to resorb their young and invariably show an early placental sign (10th or 11th day). In the earlier report[1], we stated that it was possible to palpate fetuses in those females that had been on ergot during the initial phase of pregnancy. This observation was probably incorrect since palpation of young in the initial stages of pregnancy was quite uncertain, the evidence of the present study indicating that the situation was not similar to typical resorption as in the case of pregnant vitamin E-deficient rats. It seems likely that resorption, if it occurs, must take place very early in pregnancy.

Fitzhugh *et al.*[2] give evidence that ergot when fed to rats for long periods may cause enlargement of the ovaries owing to marked hyperplasia of the corpus luteum. Examination of the internal organs was not made in the present study, but it was demonstrated that ergot, when fed for comparatively short periods to unbred females, did not alter the estrous cycle nor the reproductive ability if the ergot feed was removed at the time of breeding. However, if continued on ergot for several days after breeding they invariably failed to produce young. More research would be required to determine the actual mechanism in this reproductive failure.

The results leave little doubt that lactation in female rats can be completely stopped with ergot or ergotoxine adminstration. Resumption of lactation occurred upon removal of the drug, provided also that the young had sufficient strength remaining to nurse. One might argue that the drug found its way into the milk and thereby poisoned the young directly. However, this does not seem a very plausible explanation from the observations made in this study. In all cases where ergot or ergotoxine was administered to a female nursing a litter, it was noted that the small whitish masses in the stomach of the young representing milk intake disappeared and that for the first few days the young nursed vigorously and frequently but no milk was found in

§These litters are shown in figures 2 and 3.

‡The actual growth curves are shown in figure 1.

the stomachs. Cunningham *et al.*[3] reported that both milk and meat from ergotized cattle were nontoxic as determined by feeding experiments and chemical analysis.

Inhibition of milk secretion appeared to be different in rats as compared with brood sows. In the earlier work reported on swine[1], ergot prevented normal mammary gland development. In the case of rats, however, there was undoubtedly some milk secretion, initially, but persisted for only a short time.

There appears to be considerable species difference with respect to the effect of ergot on reproduction and lactation. Only negative results were obtained with guinea pigs[1]. Tretsven[4], of this station, has fed ergot up to 75 Gm. per day without apparent reduction of milk flow in a dairy cow. This is contrary to the experience of Petersen[5], who stated that milk secretion was reduced to 10 per cent of normal in a week in cows fed 10 Gm. of ergot daily. The writer fed a grain ration containing about 0.5 per cent ergot‖ up to 2 lb. per day for fifty days before lambing to 4 pregnant ewes. All of these ewes were shown to have good milk secretion and apparently had normal lambs.

SUMMARY

Ergot from different sources (rye, barley, wheat, and grass), when fed to bred female rats, produced similar toxic effects on reproduction and lactation. A 1.0 per cent ergot ration fed *ad libitum* to 15 females during the first ten days or longer after breeding caused resorption of the young. Five bred females, which were fed 1.0 per cent ergot beginning with the tenth day of pregnancy, produced young after a normal gestation period (22 days). However, the young were about 17 per cent below the average birth weight of a control group of 5 females. No young survived from those females continued on ergot diets after littering.

Essentially the same effects were obtained by feeding a ration containing 3 mg. per cent of ergotoxine to bred females. The results showed that this alkaloid caused resorption of young if fed to animals early in pregnancy. In the case of lactating females, ergotoxine was able to inhibit milk secretion completely. Three litters from females on ergotoxine during pregnancy and lactation failed to gain in weight and all died. Four normal litters from females previously on the stock (control) ration made no gains when 3 mg. per cent ergotoxine was added to the feed. All the young died. Three litters, at the point of death, completely recovered after ergotoxine was removed from the rations of the dams.

Ergot apparently had no effect on the estrous cycle of 4 open females on which daily examinations of vaginal smears were made. No evidence was found that the breeding ability of males was impaired by feeding 1.0 per cent ergot for a period of two months.

Both a 1.0 per cent ergot ration and a 3 mg. per cent ergotoxine ration were shown to be unpalatable.

‖This was the same ergotized barley shown to be toxic to swine and rats previously reported[1] but it was ground, one year old, and stored under ordinary farm conditions.

References

[1]Nordskog, A. W., and Clark, R. T.: Ergotism in Pregnant Sows, Female Rats, and Guinea Pigs. Am. J. Vet. Res., *6*, (1945): 107-116.

[2]Fitzhugh, O. G., Nelson, A. A., and Calvery, H. O.: Chronic Toxicity of Ergot. J. Pharmacol. and Exptl. Therap., *82*, (1944): 364-376.

[3]Cunningham, I. J., McIntosh, I. G., and Swan, J. B.: The Nontoxicity of Milk and Meat from Ergotized Cattle. New Zealand J. Sci. and Tech., *26A*, (1944): 125-136. (Cited in Biol. Abstr., *19*, (7), (1945): 54.

[4]Tretsven, J. O.: Unpublished data. Montana Agric. Exper. Sta. Bozeman, Mont. 1945.

[5]Petersen, W. E.: Private communication. Minnesota Agric. Exper. Sta., St. Paul, Minn.

AMERICAN JOURNAL OF VETERINARY RESEARCH

VOLUME VII

NUMBERS 22, 23, 24, 25

1946

COPYRIGHT 1946 BY AMERICAN VETERINARY MEDICAL ASSOCIATION

PUBLISHED BY

AMERICAN VETERINARY MEDICAL ASSOCIATION

Index of Authors

Alicata, Joseph E., 94.
Andrews, F. N., 379, 385.

Baker, James A., 142, 152, 179, 189, 196, 212.
Bartlett, D. E., 417.
Beach, B. A., 32.
Beath, O. A., 52, 57.
Beck, J. D., 470.
Brandly, C. A., 243, 250, 289, 307, 333.
Braun, W., 450.
Brewer, N. R., 123.
Brownstein, Bernard, 123.

Campbell, Hope M., 45.
Cameron, H. S., 21.
Cole, Clarence R., 69.
Cooper, H. K., 228.
Cunningham, C. H., 466.

Delez, A. L., 11, 388.
Dennis, Joe, 37.
Donham, C. R., 11, 388.

Elias, Hans, 343.

Feenstra, E. S., 432.

Getty, R., 437.
Green, W. W., 395.
Greig, A. S., 196.
Griffiths, Henry J., 135, 145, 152, 164, 170.

Hale, M. W., 199, 212, 222.
Harbaugh, F. G., 37.
Hinshaw, W. R., 62.
Huddleson, I. Forest, 5.
Hughes, Carrie Ola, 365.
Hutchings, L. M., 11, 379, 385, 388.

Jenkins, Dubois L., 135, 142, 145, 152, 164, 174, 183, 212.
Jones, E. Elizabeth, 243, 289, 307.
Jones, T. C., 403.
Jungherr, E. L., 243, 250, 289, 307, 333.

Kemper, H. E., 350.
Kosin, I. L., 477.

Lee, A. M., 41.
Lucker, John T., 101.

McCarter, Janet, 32.
McNeil, E., 62.
Mathews, Frank P., 78.
Maurer, Fred D., 145, 152, 164, 193, 212, 403.
Morgan, Banner Bill, 45.
Morrill, Don Rex, 355.
Moses, H. E., 243, 250, 289, 307, 333.
Muth, O. H., 355.

Neumayer, Eleanor M., 101.
Nordskog, A. W., 490.

Olsen, O. Wilford, 358.

Riser, W. H., 455.
Roberts, I. H., 350.
Roby, T. O., 222, 403.
Rogers, H., 481.
Rosenfeld, Irene, 52, 57.
Ryff, J .F., 41.

Shahan, M. S., 27.
Shope, Richard E., 135, 145, 152, 164, 170, 174.
Spencer, G. R., 32.
Stavitsky, A. B., 470.
Stein, C. D., 481.
Stuart, H. O., 466.

Terrence, James, 189.
Thorp, F., Jr., 432.
Tyzzer, E. E., 250.

Waletzky, Emanuel, 365.
Walker, R. V. L., 142, 145, 164, 183, 199, 212.
Wender, S. H., 486.
Willet, Elwin L., 94.

Index of Subjects

A

Activity of Sulfanilamides and Other Compounds in Avian Coccidiosis (Eimeria Tenella), The Relative, 365.
Agalactia in Ergotized Female Rats, Reproductive Failure and, 490.
Anthrax Spores from Vapors of Heated Anthrax Spore Suspensions and from the Distillate from Anthrax Spore Suspensions. Recovery of Viable, 481.
Antimony Compounds, Treatment of Filarial Dermatosis of Sheep with, 350.
Antiviral Activity via the Egg and the Rôle of Congenital Passive Immunity to Newcastle Disease in Chickens, Transmission of, 333.
Attenuated Rinderpest Vaccine Including Some Observations on the Keeping Qualities and Potency Tests, Rinderpest XIV. Immunization Experiments with, 212.
Attenuated Strain of Virus, Rinderpest XIII. The Production of Rinderpest Vaccine from an, 199.
Attenuated Virus Vaccine, Rinderpest XV. Morphological Changes in the Blood of Young Cattle during and after Vaccination with, 222.
Attenuation of Rinderpest Virus for Cattle by Cultivation in Embryonating Eggs, Rinderpest VII. The, 174.
Avian Coccidiosis (Eimeria Tenella), The Relative Activity of Sulfanilamides and Other Compounds in, 365.

B

Blood Constituents of Cows, Average Levels of Various, 450.
Blood of Dairy Cattle, The Carbon Dioxide Content of the, 37.
Blood of Young Cattle During Rinderpest and After Vaccination with Attenuated Virus Vaccine, Rinderpest XV. Morphological Changes in the, 222.
Boar, Studies on Brucellosis in Swine. III. Brucella Infection in the, 379.
Boars, Studies on Brucellosis in Swine. IV. Semen Quality in Brucella Infected, 385.
Bovine Animal during Prenatal Life, Comparative Growth of the Sheep and, 395.
Bovine Pyelonephritis, Bacteriopathology of Infectious, 432.
Bovine Tissue Vaccines, Rinderpest III. Immunization Experiments with Inactivated, 145.
Bronchitis of Chickens, The Effect of Certain Chemical Agents on the Virus of Infectious, 466.
Brucella, The Mucoid Phases of the Genus, 5.
Brucella Suis, Studies on Brucellosis of Swine II. Exposure and Reexposure Experiments with, 11.
Brucellosis of Swine II. Exposure and Reexposure Experiments with Brucella Suis, Studies on, 11.
Brucellosis in Swine. III. Brucella Infection in the Boar, Studies on, 379.
Brucellosis in Swine. IV. Semen Quality in Brucella Infected Boars, Studies on, 385.
Brucellosis of Swine IV. The Unit-Segregation System of Eradication, 21.
Brucellosis in Swine. V. Reproduction Studies with Naturally Infected Sows and Boars, 388.
Buckwheat, The Action of Photosensitizing Agents Isolated from, 486.
Bulls, Experimental Treatment of Trichomonas Foetus in, 417.
(Bunostomum Trigonocephalum) Infections in Lambs Fed an Adequate Diet, Experiments on the Pathogenicity of the Hookworm, 101.

C

Calf Diphtheria, The Etiology of, 41.
Calves to Vaccination against Rinderpest, Rinderpest IX. Neutralization Tests in Rabbits as a Measure of the Immune Responses in, 183.

Carbon Dioxide Content of the Blood of Dairy Cattle, The, 37.
Cat, The Histopathology of Panleucopenia (Agranulocytosis) in the Domestic, 455.
Cattle Associated with Leptospira, A Contagious Disease of, 78.
Cattle, The Carbon Dioxide Content of the Blood of Dairy, 37.
Cattle by Cultivation in Embryonating Eggs, Rinderpest VII. The Attenuation of Rinderpest Virus for, 174.
Cattle, The Histopathology of a Focal Hepatitis and of Its Termination ("Sawdust" and "Telang" Liver) in, 437.
Cattle During Rinderpest and After Vaccination with Attenuated Virus Vaccine, Rinderpest XV. Morphological Changes in the Blood of Young, 222.
Changes in the Equine Larynx Associated with Laryngeal Hemiplegia, 69.
Chemical Agents on the Virus of Infectious Bronchitis of Chickens, The Effect of Certain, 466.
Chemical Changes in the Tissue Following Selenium Administration, The Influence of Protein Diets on Selenium Poisoning II. The, 57.
Chemotherapy of Trichomonas Foetus (Protozoa) in Vitro, 45.
Chicken Embryo and the Chorioallantoic Membrane to Inoculation with Various Strains of Mycobacterium Paratuberculosis, Reaction of the Developing, 470.
Chickens, The Effect of Certain Chemical Agents on the Virus of Infectious Bronchitis of, 466.
Chickens against Newcastle Disease, Immunization of, 307.
Chicks Hatched from Infected Eggs, Rinderpest VI. The Persistence of Virus in, 170.
Chicks to Measure the Concentration of Rinderpest Virus Propagated in Eggs, Rinderpest XII. The Successful Use of Young, 196.
Coccidiosis, Observations on the Prophylactic and Curative Value of Sulfaguanidine in Swine, 94.
Complement Fixation Test for Rinderpest, Rinderpest XVI. 228.
Concentrations of Rinderpest Virus Propagated in Eggs, Rinderpest XII. The Successful Use of Young Chicks to Measure the, 196.
Contagious Disease of Cattle Associated with Leptospira, A, 78.
Cows, Average Levels of Various Blood Constituents of, 450.
Crystal Violet on Vesicular Stomatitis Virus, Effect of Temperature, Phenol, and, 27.
Cultivation of Rinderpest Virus in the Developing Hen's Egg, Rinderpest I. The, 135.
Curative Value of Sulfaguanidine in Swine Coccidiosis, Observations on the Prophylactic and, 94.

D

Developing Hen's Egg, Rinderpest I. The Cultivation of Rinderpest Virus in the, 135.
Developing Hens' Eggs, Rinderpest IV. Infection of the Embryos and the Fluids of, 152.
Diet, Experiments on the Pathogenicity of the Hookworm (Bunostomun Trigonocephalum) Infections in Lambs Fed an Adequate, 101.
Diphtheria, The Etiology of Calf, 41.
Disease of Cattle Associated with Leptospira, A Contagious, 78.
Distillate from Anthrax Spore Suspensions, Recovery of Viable Anthrax Spores from Vapors of Heated Anthrax Spore Suspensions and from the, 481.

E

Egg-Cultivated Virus, Rinderpest V. Attempts to Prepare an Effective Rinderpest Vaccine from Inactivated, 164.
Egg, Rinderpest I. The Cultivation of Rinderpest Virus in the Developing Hen's, 135.
Eggs, Rinderpest VI. The Persistence of Virus in Chicks Hatched from Infected, 170.
Eggs, Rinderpest VII. The Attenuation of Rinderpest Virus for Cattle by Cultivation in Embryonating, 174.
Eggs, Rinderpest XII. The Successful Use of Young Chicks to Measure the Concentration of Rinderpest Virus Propagated in, 196.
(Eimeria Tenella) The Relative Activity of Sulfanilamides and Other Compounds in Avian Coccidiosis, 365.
Embryonating Eggs, Rinderpest VII. The Attenuation of Rinderpest Virus for Cattle by Cultivation in, 174.
Embryos and the Fluids of Developing Hens' Eggs, Rinderpest IV. Infection of the, 152.
Enterotoxemia (Pulpy Kidney Disease) in Lambs by the Use of Alum Precipitated Toxoid, Control of, 355.
Epizoötiology of Newcastle Disease of Poultry, 243.
Equine Larynx Associated with Laryngeal Hemiplegia, Changes in the, 69.
Equine Periodic Ophthalmia, The Relation of Riboflavin to, 403.
Ergotized Female Rats, Reproductive Failure and Agalactia in, 490.
Etiology of Calf Diphtheria, The, 41.

F

Fellowships Available, Research, 384.
Filarial Dermatosis of Sheep with Antimony Compounds, Treatment of, 350.
Fixation Test for Rinderpest, Rinderpest XVI. Complement, 228.
Fluids of Developing Hens' Eggs, Rinderpest IV. Infection of the Embryos and the, 152.
Fowl Plague and Newcastle Disease, The Comparative Pathology of, 250.
Fowl, The Transmission of Lymphomatosis in the, 123.

G

Galapagos Turtles, a Gila Monster, and an Iguana, Salmonella from, 62.
Gila Monster, and an Iguana, Salmonella from Galapagos Turtles, a, 62.
Guinea Pigs to the Virus of Rinderpest, Rinderpest X. The Response of, 189.

H

Hemiplegia, Changes in the Equine Larynx Associated with Laryngeal, 69.
Hen's Egg, Rinderpest I. The Cultivation of Rinderpest Virus in the Developing, 135.
Hepatitis and of Its Termination ("Sawdust" and "Telang" Liver) in Cattle, The Histopathology of a Focal, 437.
Hexachlorethane-Bentonite Suspension for the Removal of the Common Liver Fluke, Fasciola Hepatica, from Sheep, 358.
Hookworm (Bunostomum Trigonocephalum) Infections in Lambs Fed an Adequate Diet, Experiments on the Pathogenicity of the, 101.
Horse, The Tonsils of the, 343.

I

Iguana, Salmonella from Galapagos Turtles, a Gila Monster, and an, 62.
Immune Responses in Calves to Vaccination against Rinderpest, Rinderpest IX. Neutralization Tests in Rabbits as a Measure of the, 183.
Immunity to Newcastle Disease in Chickens, Transmission of Antiviral Activity via the Egg and the Rôle of Congenital Passive, 333.
Immunity Reactions, Rinderpest II. Certain, 142.
Immunization of Chickens against Newcastle Disease, 307.

Immunization Experiments with Attenuated Rinderpest Vaccine Including Some Observations on the Keeping Qualities and Potency Tests, Rinderpest XIV, 212.
Immunization Experiments with Inactivated Bovine Tissue Vaccines, Rinderpest III. 145.
Inactivated Egg-Cultivated Virus, Rinderpest V. Attempts to Prepare an Effective Rinderpest Vaccine from, 164.
Inactivated Bovine Tissue Vaccines, Rinderpest III. Immunization Experiments with, 145.
Infected Eggs, Rinderpest VI. The Persistence of Virus in Chicks Hatched from, 170.
Infection of the Embryos and the Fluids of Developing Hens' Eggs, Rinderpest IV. 152.
Infection of Streptococcus Agalactiae, Reservoirs of, 32.
Influence of Protein Diets on Selenium Poisoning. I., The, 52.
Influence of Protein Diets on Selenium Poisoning. II. The Chemical Changes in the Tissues Following Selenium Administration, The, 57.
Isolation and Identification of Newcastle Disease Virus, 289.

J

Journal Changes Its Make-Up, The, 10.

K

Keeping Qualities and Potency Tests, Rinderpest XIV. Immunization Experiments with Attenuated Rinderpest Vaccine Including Some Observations on the, 212.

L

Lambs Fed an Adequate Diet, Experiments on the Pathogenicity of the Hookworm (Bunostomum Trigonocephalum) Infections in, 101.
Lambs by the Use of Alum Precipitated Toxoid, Control of Enterotoxemia (Pulpy Kidney Disease) in, 355.
Laryngeal Hemiplegia, Changes in the Equine Larynx Associated with, 69.
Leptospira, A Contagious Disease of Cattle Associated with, 78.
Liver Fluke, Fasciola Hepatica, from Sheep, Hexachlorethane-Bentonite Suspension for the Removal of the Common, 358.
Lymphomatosis in the Fowl, The Transmission of, 123.

M

Make-Up, The Journal Changes Its, 10.
Mediums, Rinderpest XI. The Survival of Rinderpest in Various 193.
Morphological Changes in the Blood of Young Cattle During Rinderpest and After Vaccination with Attenuated Virus Vaccine, Rinderpest XV. 222.
Mucoid Phases of the Genus Brucella, The, 5.
Mycobacterium Paratuberculosis, Reaction of the Developing Chicken Embryo and the Chorioallantoic Membrane to Inoculation with Various Strains of, 470.

N

Neutralization Tests in Rabbits as a Measure of the Immune Responses in Calves to Vaccination against Rinderpest, Rinderpest IX. 183.
Newcastle Disease in Chickens, Transmission of Antiviral Activity via the Egg and the Rôle of Congenital Passive Immunity to, 333.
Newcastle Disease, The Comparative Pathology of Fowl Plague and, 250.
Newcastle Disease, Immunization of Chickens against, 307.
Newcastle Disease of Poultry, Epizoötiology of, 243.
Newcastle Disease Virus, Isolation and Identification of, 289.

O

Ophthalmia, The Relation of Riboflavin to Equine Periodic, 403.

P

Panleucopenia (Agranulocytosis) in the Domestic Cat, The Histopathology of, 455.
Pathogenicity of the Hookworm (Bunostomum Trigonocephalum) Infections in Lambs Fed an Adequate Diet, Experiments on the, 101.
Pathology of Fowl Plague and Newcastle Disease, The Comparative, 250.
Persistence of Virus in Chicks Hatched from Infected Eggs, Rinderpest VI. The, 170.
Phenol, and Crystal Violet on Vesicular Stomatitis Virus, Effect of Temperature, 27.
Photosensitizing Agents Isolated from Buckwheat, The Action of, 486.
Poisoning II. The Chemical Changes in the Tissues Following Selenium Administration, The Influence of Protein Diets on Selenium, 57.
Poisoning. I., The Influence of Protein Diets on Selenium, 52.
Potency Tests, Rinderpest XIV. Immunization Experiments with Attenuated Rinderpest Vaccine Including Some Observations on the Keeping Qualities and, 212.
Poultry, Epizoötiology of Newcastle Disease of, 243.
Poultry, Two Examples of Terata in the Caudal Region of Domestic, 477.
Prenatal Life, Comparative Growth of the Sheep and Bovine Animal during, 395.
Production of Rinderpest Vaccine from an Attenuated Strain of Virus, Rinderpest XIII. The, 199.
Prophylactic and Curative Value of Sulfaguanidine in Swine Coccidiosis, Observations on the, 94.
Protein Diets on Selenium Poisoning. I., The Influence of, 52.
Protein Diets on Selenium Poisoning II. The Chemical Changes in the Tissues Following Selenium Administration, The Influence of, 57.
(Protozoa) in Vitro, Chemotherapy of Trichomonas Foetus, 45.
Pyelonephritis, Bacteriopathology of Infectious Bovine, 432.

R

Rabbits as a Measure of the Immune Responses in Calves to Vaccination against Rinderpest, Rinderpest IX. Neutralization Tests in, 183.
Rabbits, Rinderpest VIII. Rinderpest Infection in, 179.
Rats. Reproductive Failure and Agalactia in Ergotized Female, 490.
Reactions, Rinderpest II. Certain Immunity, 142.
Reproduction Studies with Naturally Infected Sows and Boars, Brucellosis in Swine. V., 388.
Reproductive Failure and Agalactia in Ergotized Female Rats, 490.
Research Fellowships Available, 384.
Reservoirs of Infection of Streptococcus Agalactiae, 32.
Response of Guinea Pigs to the Virus of Rinderpest, Rinderpest X. The, 189.
Riboflavin to Equine Periodic Ophthalmia, The Relation of, 403.
Rinderpest I: The Cultivation of Rinderpest Virus in the Developing Hen's Egg, 135.
Rinderpest II: Certain Immunity Reactions, 142.
Rinderpest III: Immunization Experiments with Inactivated Bovine Tissue Vaccines, 145.

Rinderpest IV: Infection of the Embryos and the Fluids of Developing Hens' Eggs, 152.
Rinderpest V: Attempts to Prepare an Effective Rinderpest Vaccine from Inactivated Egg-Cultivated Virus, 164.
Rinderpest VI: The Persistence of Virus in Chicks Hatched from Infected Eggs, 170.
Rinderpest VII: The Attenuation of Rinderpest Virus for Cattle by Cultivation in Embryonating Eggs, 174.
Rinderpest VIII: Rinderpest Infection in Rabbits, 179.
Rinderpest IX: Neutralization Tests in Rabbits as a Measure of the Immune Responses in Calves to Vaccination against Rinderpest, 183.
Rinderpest X: The Response of Guinea Pigs to the Virus of Rinderpest, 189.
Rinderpest XI: The Survival of Rinderpest in Various Mediums, 193.
Rinderpest XII: The Successful Use of Young Chicks to Measure the Concentration of Rinderpest Virus Propagated in Eggs, 196.
Rinderpest XIII: The Production of Rinderpest Vaccine from an Attenuated Strain of Virus, 199.
Rinderpest XIV: Immunization Experiments with Attenuated Rinderpest Vaccine Including Some Observations on the Keeping Qualities and Potency Tests, 212.
Rinderpest XV: Morphological Changes in the Blood of Young Cattle During Rinderpest and After Vaccination with Attenuated Virus Vaccine, 222.
Rinderpest XVI: Complement Fixation Test for Rinderpest, 228.

S

Salmonella from Galapagos Turtles, a Gila Monster, and an Iguana, 62.
("Sawdust" and "Telang" Liver) in Cattle, The Histopathology of a Focal Hepatitis and of Its Termination, 437.
Segregation System of Eradication, Brucellosis of Swine IV. The Unit-, 21.
Selenium Administration, The Influence of Protein Diets on Selenium Poisoning II. The Chemical Changes in the Tissues Following, 57.
Selenium Poisoning. I., The Influence of Protein Diets on, 52.
Semen Quality in Brucella Infected Boars, Studies on Brucellosis in Swine. IV., 385.
Sheep with Antimony Compounds, Treatment of Filarial Dermatosis of, 350.
Sheep and Bovine Animal during Prenatal Life, Comparative Growth of the, 395.
Sheep, Hexachlorethane-Bentonite Suspension for the Removal of the Common Liver Fluke, Fasciola Hepatica, from, 358.
Sows and Boars, Brucellosis in Swine. V. Reproduction Studies with Naturally Infected, 388.
Stomatitis Virus, Effect of Temperature, Phenol, and Crystal Violet on Vesicular, 27.
Streptococcus Agalactiae, Reservoirs of Infection of, 32.
Sulfaguanidine in Swine Coccidiosis, Observations on the Prophylactic and Curative Value of, 94.
Sulfanilamides and Other Compounds in Avian Coccidiosis (Eimeria Tenella), The Relative Activity of, 365.
Survival of Rinderpest in Various Mediums, Rinderpest XI. The, 193.
Swine Coccidiosis, Observations on the Prophylactic and Curative Value of Sulfaguanidine in, 94.
Swine II. Exposure and Reexposure Experiments with Brucella Suis, Studies on, Brucellosis of, 11.
Swine III. Brucella Infection in the Boar, Studies on Brucellosis in, 379.
Swine IV. The Unit-Segregation System of Eradication, Brucellosis of, 21.
Swine IV. Semen Quality in Brucella Infected Boars. Studies on Brucellosis in, 385.
Swine V. Reproduction Studies with Naturally Infected Sows and Boars, Brucellosis in, 388.

T

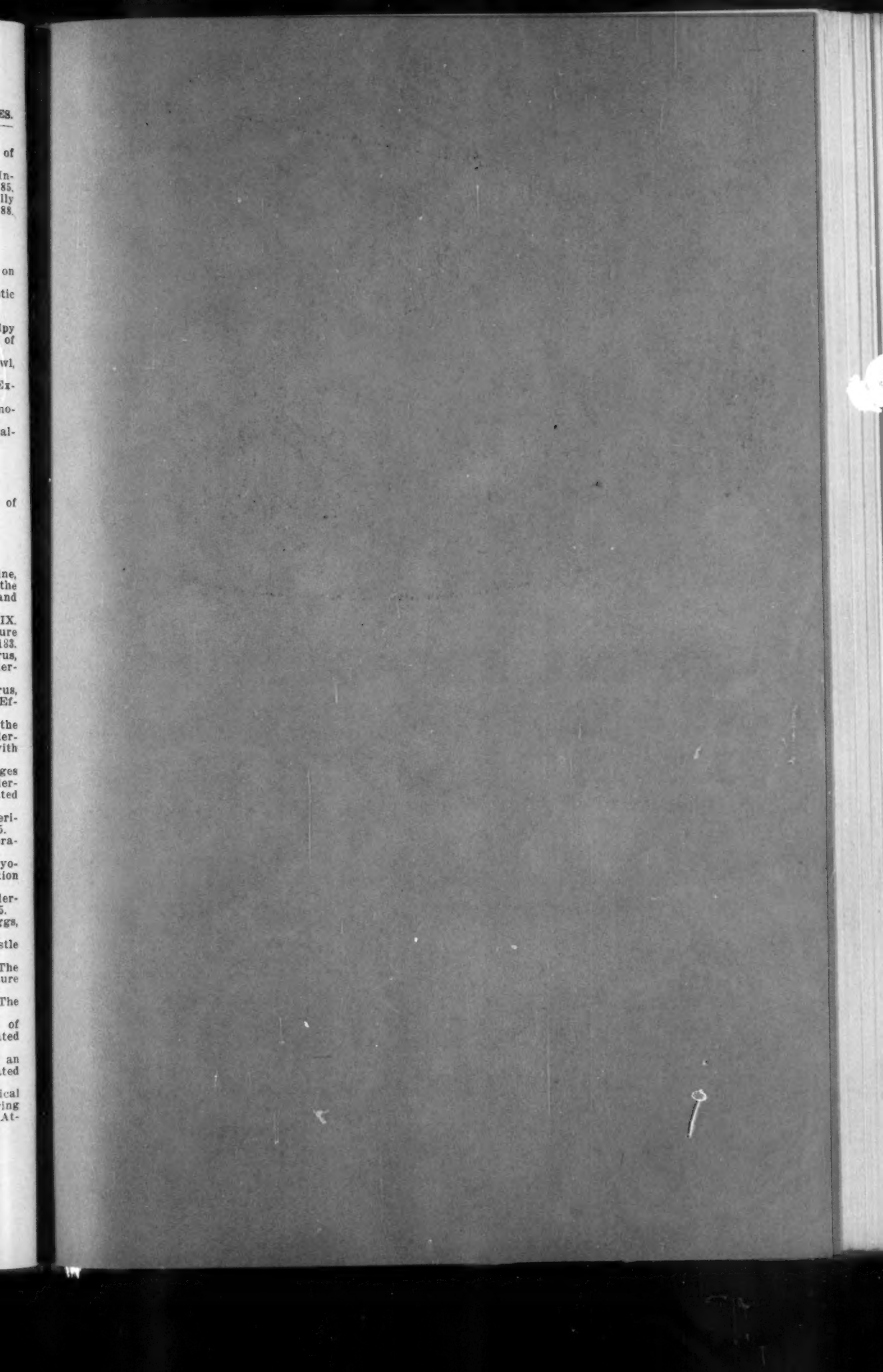
Temperature, Phenol, and Crystal Violet on Vesicular Stomatitis Virus, Effect of, 27.
Terata in the Caudal Region of Domestic Poultry, Two Examples of, 477.
Tonsils of the Horse, The, 343.
Toxoid, Control of Enterotoxemia (Pulpy Kidney Disease) in Lambs by the Use of Alum Precipitated, 355.
Transmission of Lymphomatosis in the Fowl, The, 123.
Trichomonas Foetus Infection in Bulls, Experimental Treatment of, 417.
Trichomonas Foetus (Protozoa) in Vitro, Chemotherapy of, 45.
Turtles, a Gila Monster, and an Iguana, Salmonella from Galapagos, 62.

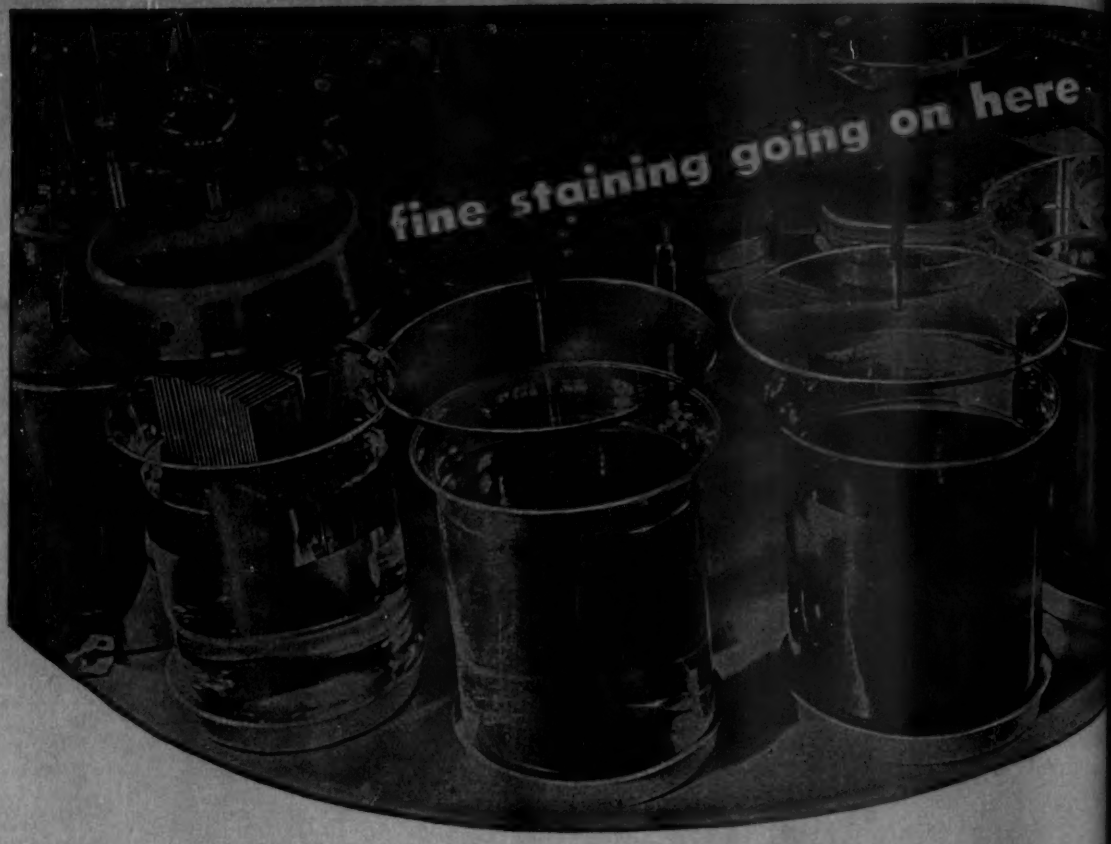
U

Unit-Segregation System of Eradication of Brucellosis of Swine IV., 21.

V

Vaccination with Attenuated Virus Vaccine, Rinderpest XV. Morphological Changes in the Blood of Young Cattle during Rinderpest and after, 222.
Vaccination against Rinderpest, Rinderpest IX. Neutralization Tests in Rabbits as a Measure of the Immune Responses in Calves to, 183.
Vaccine from an Attenuated Strain of Virus, Rinderpest XIII. The Production of Rinderpest, 199.
Vaccine from Inactivated Egg-Cultivated Virus, Rinderpest V. Attempts to Prepare an Effective Rinderpest, 164.
Vaccine Including Some Observations on the Keeping Qualities and Potency Tests, Rinderpest XIV. Immunization Experiments with Attenuated Rinderpest, 212.
Vaccine, Rinderpest XV. Morphological Changes in the Blood of Young Cattle During Rinderpest and After Vaccination with Attenuated Virus, 222.
Vaccines, Rinderpest III. Immunization Experiments with Inactivated Bovine Tissue, 145.
Vesicular Stomatitis Virus, Effect of Temperature, Phenol, and Crystal Violet on, 27.
Virus for Cattle by Cultivation in Embryonating Eggs, Rinderpest VII. The Attenuation of Rinderpest, 174.
Virus in the Developing Hen's Egg, Rinderpest I. The Cultivation of Rinderpest, 135.
Virus in Chicks Hatched from Infected Eggs, Rinderpest VI. The Persistence of, 170.
Virus, Isolation and Identification of Newcastle Disease, 289.
Virus Propagated in Eggs, Rinderpest XII. The Successful Use of Young Chicks to Measure the Concentration of Rinderpest, 196.
Virus of Rinderpest, Rinderpest X. The Response of Guinea Pigs to the, 189.
Virus, Rinderpest XIII. The Production of Rinderpest Vaccine from an Attenuated Strain of, 199.
Virus, Rinderpest V. Attempts to Prepare an Effective Rinderpest Vaccine from Inactivated Egg-Cultivated, 164.
Virus Vaccine, Rinderpest XV. Morphological Changes in the Blood of Young Cattle During Rinderpest and After Vaccination with Attenuated, 222.





This is a close-up view of a rack of microslides undergoing staining on the **Autotechnicon.** The process is *completely automatic,* and geared to split-second timing. The slides are now descending into one staining-fluid beaker: at the precise moment when the required immersion period has been completed, they will be promptly raised, and rapidly shifted to the next station in the sequence . . . automatically, infallibly. During immersion the slides are rotated gently within the fluid, thus assuring rapid, even stain permeation.

Any technique prescribed by the pathologist may be employed. Once the master dial has been set for the correct time intervals, the method may be repeated time after time, without the minutest variation, and independent of any supervision. Each batch of slides put through is stained in *exactly* the same way, resulting in unvarying uniformity.

Autotechnicon staining is not only convenient, dependable, time-saving . . . it produces "Sunday-best" slides . . . *every day.*

Autotechnicon

automatic tissue processing . . . fixation, dehydration, infiltration, washing, staining

THE TECHNICON COMPANY
215 East 149th Street • New York 51, N. Y.

F601
43

American Journal of Veterinary Research

UNIVERSITY OF CALIFORNIA
MAR 7 1947
AGRI

CONTENTS

The Chemotherapy of Calf Pneumonia. I. Some Pharmacologic Aspects of Sulfonamide Administration to Normal Calves 1
L. Meyer Jones

The Chemotherapy of Calf Pneumonia. II. The Use of Sulfathiazole, Sulfapyridine, and Sulfadiazine in the Treatment of Calf Pneumonia 14
L. Meyer Jones

The Genesis of Bovine Udder Infection and Mastitis. II. The Occurrence of Streptococcal Infection in a Cow Population During a Seven-Year Period and Its Relationship to Age 29
James M. Murphy

Infection of the Bovine Udder with Yeastlike Fungi 43
James M. Murphy and Charles H. Drake

Submucosal Glands in the Bovine Ileum 52
Hans Elias

Vaccination Studies on Bovine Trichomoniasis 54
Banner Bill Morgan

Some Experimental Investigations on the Etiology of Leucemia in Cattle 57
J. Egehoj

Length of Gestation in Range Sheep 66
Clair E. Terrill and L. N. Hazel

Studies in Baby Pig Mortality. IV. Chemistry of the Blood During Fasting and Refeeding of Weanling Pigs 73
Vera M. Hamawalt and Jesse Sampson

The Behavior of the Peripheral Blood Elements in Panleucopenia (Agranulocytosis) of the Domestic Cat 82
Wayne H. Riser

Chicken Embryo Vaccine Against Newcastle Disease [Pneumoencephalitis] 91
Ramon A. Acevedo and Isidro L. Mendoza

Studies in Avian Leucosis. I. The Transmissibility of Visceral Lymphomatosis ... 103
Olive Stull Davis and L. P. Doyle

Studies in Avian Leucosis. II. The Use of Biopsy Technique in the Study of Visceral Lymphomatosis 113
Olive Stull Davis and L. P. Doyle

Adsorbate Newcastle Disease (Avian Pest) Vaccine 120
Anacleto B. Coronel

Immunity and Cross Immunity Reactions Obtained with Several Avian Lymphoid Tumor Strains 128
B. R. Burmester and T. C. Belding

Isolation of Shigella Gallinarum from Turkey Eggs 133
W. A. Boney

Transmission of the Virus of Newcastle Disease to the Syrian Hamster 136
Reginald L. Reagan, Mary G. Lillie, Leo J. Poelma, and A. L. Brueckner

Infection and Transmission of Brucella by Ectoparasites 138
Raul M. Tovar

A Publication of the American Veterinary Medical Association

Volume VIII JANUARY, 1947 Number 26

Consecutive order of appearance, rather than identity with any given volume, is the plan adopted for numbering the issues of the American Journal of Veterinary Research. Following are the volume and issue numbers published to date.

VOL. I, No. 1—OCTOBER, 1940

VOL. II, No. 2—JANUARY, 1941	VOL. V, No. 14—JANUARY, 1944
VOL. II, No. 3—APRIL, 1941	VOL. V, No. 15—APRIL, 1944
VOL. II, No. 4—JULY, 1941	VOL. V, No. 16—JULY, 1944
VOL. II, No. 5—OCTOBER, 1941	VOL. V, No. 17—OCTOBER, 1944
VOL. III, No. 6—JANUARY, 1942	VOL. VI, No. 18—JANUARY, 1945
VOL. III, No. 7—APRIL, 1942	VOL. VI, No. 19—APRIL, 1945
VOL. III, No. 8—JULY, 1942	VOL. VI, No. 20—JULY, 1945
VOL. III, No. 9—OCTOBER, 1942	VOL. VI, No. 21—OCTOBER, 1945
VOL. IV, No. 10—JANUARY, 1943	VOL. VII, No. 22—JANUARY, 1946
VOL. IV, No. 11—APRIL, 1943	VOL. VII, No. 23—APRIL, 1946 (PART 1)
	VOL. VII, No. 23—APRIL, 1946 (PART 2)
VOL. IV, No. 12—JULY, 1943	VOL. VII, No. 24—JULY, 1946
VOL. IV, No. 13—OCTOBER, 1943	VOL. VII, No. 25—OCTOBER, 1946

VOL. VIII, No. 26—JANUARY, 1947

COPYRIGHT 1947 BY AMERICAN VETERINARY MEDICAL ASSOCIATION

Published quarterly at 600 S. Michigan Ave., Chicago, Ill., by the American Veterinary Medical Association. Entered as Second-Class Matter January 31, 1941, at the Post Office at Chicago, Illinois, under the Act of March 3, 1879. Subscription price: $4.00 per year for United States, Canada, and countries affiliated with the Pan American Postal Union; foreign countries, $4.50. Members of the American Veterinary Medical Association may subscribe to the American Journal of Veterinary Research for $2.50 per year added to the annual dues of $7.00. Single copies $1.25. Address all correspondence to American Veterinary Medical Association.

Printed in the U. S. A.

In Place of Tincture of Benzoin

For
Skin Protection

NEW! Now Available In TUBES

SEALSKIN

LIQUID PLASTIC
SKIN ADHESIVE

Pat. Applied For

SEALSKIN is a hypo-allergenic LIQUID PLASTIC SKIN ADHESIVE that dries to a strong yet soft elastic COHESIVE film which adheres to the skin and dressings. The film is waterproof and resistant to the action of body fluids, acids, etc.

Simply apply Sealskin all around the edges of the bandage. Apply the bandage to the area.

Use 3 Ways

★ **SEALSKIN** to adhere dressings or bandages to the skin —wound dressings—skin traction bandages, etc.

★ **SEALSKIN** to prevent adhesive plaster skin reactions. Apply a protective coating to the skin before applying adhesive plaster. It peels off with the plaster leaving no debris.

★ **SEALSKIN** to prevent excoriation of the tissue in cases of draining fistulae, colostomies and the like.

J-500	Per 4 oz. tube	$1.50
J-502	Per 16 oz. jar	$3.75

Write for literature on your letterhead, please.
Order from your surgical supply dealer.

CLAY-ADAMS CO INC.

44 EAST 23rd STREET, NEW YORK 10, N. Y.